Studies on the coastal water quality in relation to the health of green mussel, Perna viridis (Linnaeus)

Thesis submitted to the
Mangalore University
for the degree of

Doctor of Philosophy

In

Biosciences

By

Geetha Sasikumar, ***M.F.Sc. (Aquaculture)***

Department of Post-graduate Studies and Research in Biosciences
Mangalore University, Mangalagangothri - 574 199,
Karnataka, India.

July, 2007

To my son Sidharth &
daughter Sangita

MANGALORE UNIVERSITY
Mangalagangothri - 574199 (India)

ಮಂಗಳೂರು ವಿಶ್ವವಿದ್ಯಾನಿಲಯ
ಮಂಗಳಗಂಗೋತ್ರಿ - ೫೭೪೧೯೯

DEPARTMENT OF POST-GRADUATE STUDIES AND RESEARCH IN BIOSCIENCES

Prof. (Dr.) M. Krishnamurthy

CERTIFICATE

*This is to certify that this thesis entitled "**Studies on the coastal water quality in relation to the health of green mussel, Perna viridis (Linnaeus)**" submitted by Ms. Geetha Sasikumar to the Mangalore University for the Degree of Doctor of Philosophy in Biosciences is based on the results of experiments and investigations carried out independently by her under my guidance and supervision at Mangalore University. The thesis or part thereof has not previously been presented for any degree, diploma, associateship, fellowship or other similar title in any University.*

Mangalagangothri

(M. Krishnamurthy)

Declaration

*I hereby declare that this thesis entitled "**Studies on the coastal water quality in relation to the health of green mussel, Perna viridis (Linnaeus)**" embodies the results of bona fide research work carried out by me under the guidance and supervision of **Dr. M. Krishnamurthy**, Professor, Department of Post-graduate Studies and Research in Biosciences, Mangalore University and that the thesis or part thereof has not previously been presented for any degree, diploma, associateship, fellowship or other similar title in any other University or Academic Institution.*

Mangalagangothri

Geetha Sasikumar

Acknowledgement

I wish to place on record, my deep sense of gratitude to all those, who have contributed in one way or the other, to the successful completion of my research work and preparation of this thesis. I am fortunate to have benefited from the valuable guidance and constant encouragements of my research supervisor, Prof. (Dr.) M. Krishnamurthy, Department of Post-graduate Studies and Research in Biosciences, Mangalore University. His continuous support throughout the course of the study helped me beyond words, in the conduct of my research programmes and preparation of the thesis. I am grateful to Prof. (Dr.) K.R. Sridhar, Chairman, Prof. (Dr.) M. Jayaram, Hegde, (Rtd.) former Chairman, and Prof. (Dr.) M. Rajashekhar, former Chairman, Department of Post-graduate Studies and Research in Biosciences for their kind support in the conduct of this study.

I wish to express my warm and sincere thanks to Prof. (Dr.) Mohan Joseph Modayil, Director, Central Marine Fisheries Research Institute, Kochi for granting me study leave to pursue the Ph.D. degree programme. I am greatly indebted to Dr. C. Muthiah, Scientist-in-Charge, Mangalore Research Centre of CMFRI, Mangalore, for his constant encouragements and constructive criticisms during the preparation of the thesis. I am deeply grateful to Dr. P.K. Krishnakumar, Sr. Scientist, V.R.C. of CMFRI, Veraval for his valuable guidance and intellectual support throughout this work. I am greatly indebted to Dr. K.S. Mohamed, Head, Molluscan Fisheries Division and Dr. K.K. Appukuttan former Head, Molluscan Fisheries Division, CMFRI, Kochi for their valuable suggestions in various aspects of the study.

During this work, I have collaborated with many colleagues, for whom, I have great regard and I wish to extend my sincere thanks to all those who have helped me in my work. I am greatly indebted to the valuable help rendered by S/Shri. G.S. Bhat, G. Sampathkumar, D. Nagaraja, Y. Muniyappa, G.D. Nataraja, B. Shridhara, S. Kemparaju, H.S. Mahadevaswamy, L.K. Suvarna, A. Keshava, Mohan S. Puthran, Smt. Uma S. Bhat and Smt. Alli C. Gupta of the M.R.C. of CMFRI, Mangalore during my research work.

My sincere thanks are due to Dr. Prathiba Rohit, Sr. Scientist, V.R.C. of CMFRI, Vishakapatnam and Dr. P.U. Zacharia, T.R.C. of CMFRI, Tuticorin for their valuable advices and friendly encouragements. Also, I am grateful to all of my Senior colleagues, Dr. N. Ramachandran, Dr. M. Vijayakumaran, Shri. S.G. Raje and Dr. A.P. Dineshbabu and colleagues, Smt. Sujitha Thomas and Dr. P.S. Swathilekshmi of M.R.C. of CMFRI, Mangalore for their sincere support and co-operation. My thanks are also due to Shri. T. Harish Nayak, Shri. Anoop A. Krishnan and Shri. Raveendra N. Durgekar, Research Scholars, M.R.C. of CMFRI, Mangalore for their help during the study.

I am greatly indebted to Dr. K.V. Lalitha, Principal Scientist, Central Institute of Fisheries Technology (CIFT), Kochi for the prudent suggestions in

framing the methodology for microbial analysis and also for critically going through the microbiology part of the thesis. I would also like to gratefully acknowledge Dr. T.V. Sankar, Senior Scientist, CIFT, Kochi for the help rendered in the analysis of organochlorines and also for his valuable comments on the thesis. I am grateful to Dr. Praveen Putran, Senior Scientist, Smt. Bindu J., Scientist (Sr. Scale) and Dr. Usha Rani, Technical Officer, CIFT, Kochi for the help rendered during the study.

I wish to extend my sincere thanks to Shri. Alexander Thomas, Deputy Director, Quality Control, Marine Products Export Development Authority of India, Kochi, for his valuable help during the course of this study. I wish to thank Shri. T.M. Sankaran, former Head, Department of Management Studies, College of Fisheries, Panangad for his guidance in statistical analysis. I would also like to thank Dr. I.S. Bright Singh, Director, School of Environmental Studies, CUSAT, Kochi for his encouragements.

I am grateful to Shri. A.C. Dinesh, Senior Geologist, Geological Survey of India, Marine Wing, Mangalore, for the help rendered in the preparation of the location maps.

I would like to gratefully acknowledge the help of Mr. U. Tharanath and all non-teaching staff of the Department of Post-graduate Studies and Research in Biosciences, Mangalore University for their kind co-operation.

I owe a great deal to Shri. Ashoka of Someshwara and Shri. M.A. Rasheed of Surathkal, who kindly helped me in sample collection even in inclement weather conditions.

Thanks to my parents, who were the driving force behind all my achievements and who always supported and helped me with unending practical chores. I owe my sincere gratitude to my husband, Sasikumar for his constant support and encouragements throughout the course of this work. Above all, I acknowledge my daughter Sangita and son Sidharth for their endurance during the course of this work. My special thanks are due to my brother-in-law Prof. P.K. Raveendran, my brother Remeyn, sisters Deepa and Smarty for their loving support.

Geetha Sasikumar

Contents

Chapter 1

General Introduction

Seafood and aquaculture industry are facing many challenges as it enters the 21st century. Global competition, strict quality regulations and complex trade policies are some of the challenges that the industry is trying to cope with. In recent years, geared by the shortage of supply against an increasing demand, many of the non-conventional seafood resources have gained considerable attention. Besides the increased exploitation of non-conventional resources, aquaculture practices are being adopted in a big way to reduce the gap between demand and supply of seafood products.

Molluscan fisheries, constituted by bivalves, gastropods and cephalopods accounted 11.6% of the world aquatic production in 2004 (FAO, FISHSTAT). Bivalves, represented by clams, oysters and mussels contributed 13.9 million tonnes to the world molluscan production (18.1 million tonnes). Among the bivalves, mussels occupy a prominent position contributing 14.7% to the world bivalve production in 2004. Mussels are traded as live, fresh, chilled, frozen, canned, dried, salted, blanched and other value added forms. In 2004 the export of mussels to various international markets accounted for USD 536.7 million.

Mussels are widely distributed in tropical and temperate conditions. They are seen typically attached to hard substratum with their byssus threads, in the intertidal and subtidal zones (Lee, 1985). Countries where mussel fishery is popular are Denmark, Italy, UK and USA. World mussel production from capture and culture fisheries recorded an increasing trend from 0.16 million tonnes in 1950 to 2.05 million tonnes in 2004 (Fig. 1.1) registering an annual increment of ~5%. This phenomenal increase (+36% since 1990) in total mussel production over the past few decades is a result of wider adoption of culture practices. Major species of mussels contributing to the global production are the

blue mussel, *Mytilus edulis* and the Mediterranean mussel, *Mytilus galloprovincialis*. China is the world's largest mussel producer contributing 33% to the total production followed by Spain, Thailand, Italy and Denmark (Fig. 1.2).

Mussel farming has a long history that dates back to the thirteenth century. Recent years witnessed significant improvements in the husbandry as a result of research and developments inspired by an increasing market demand. Consequently the global aquaculture production of mussels exceeded the harvest from natural resources by several folds reaching a proportion as high as 85% of the total mussel production in 2004. Countries where commercial mussel farming is popular are China, Spain, Thailand, New Zealand, Chile, France and Netherlands.

The green mussel, *Perna viridis* (Linnaeus, 1758) belonging to the family Mytilidae is distributed in its native tropical waters of the Indo-Pacific region of Asia (Siddall, 1980). Apart from being an important candidate species for mariculture, green mussel forms an important fishery within its distributional area. Thailand and Philippines are the major green mussel producers followed by Malaysia and Singapore (Fig. 1.3).

Bivalve production in India was estimated at 62,935 t in 2004 of which clams, mussels and oysters contributed to 50,970, 11,319 and 333 t respectively. Mussels are one of the important groups of bivalves, the natural resources of which are still underexploited in many parts of the country. Mussel production in India showed considerable increase from 4,197 t in 1990s to 11,319 t in 2004 (Kripa and Appukuttan, 2003). This increase in production is consequent to the increased demand for seafood against dwindling catches of commercially important fishes that persuaded fishermen to look for the underexploited resources. Though there is an active trade of bivalves internationally, exports from India during 2004 accounted to a meagre figure of USD 0.03 million.

Mussel fishery in India is represented by two species, the green mussel *Perna viridis* and the brown mussel *Perna indica*. Green mussel which forms extensive beds along the rocky shores of the sub-continent is the major exploited species offering a seasonal fishery of sizeable magnitude. *P. viridis* enjoys a wider distribution from Kollam to Gulf of Kutch along the west coast, (Nayar and Rao, 1985) and along the east coast, it occurs at a few places such as Visakhapatnam, Kakinada and Chennai (Fig. 1.4). The green mussel offers good potential for culture due to its adaptability to the culture conditions, wide distribution and simplicity of the farming methods. Mussel farming has been taken up in many parts of the country as a means of rural employment and as an alternate occupation for the traditional fishermen communities (Appukuttan *et al.*, 2000). Many of such activities are still in pilot scale and future development of the sector has to be meticulously planned with a perspective addressing the challenges to make it a successful rural enterprise.

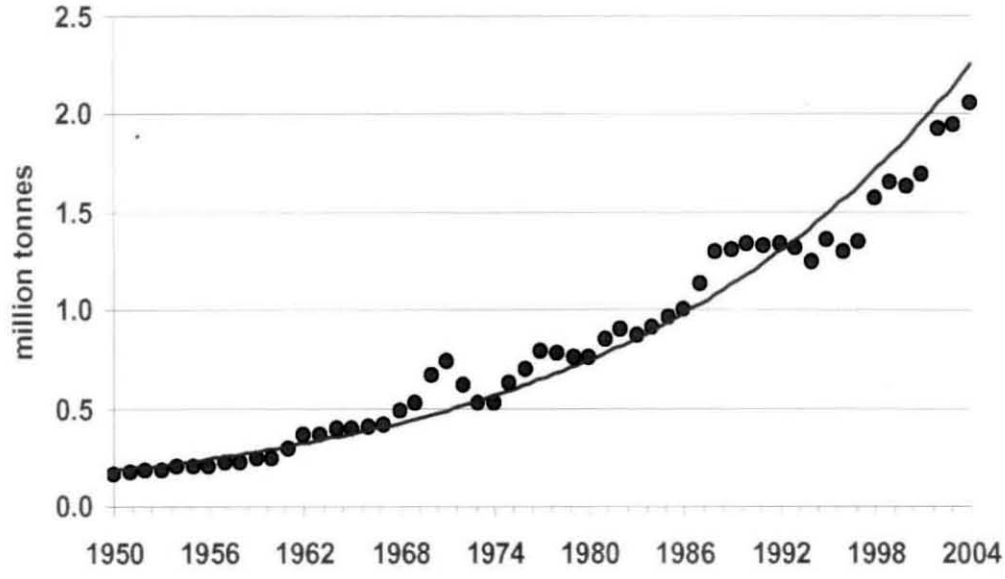

Fig. 1.1. World mussel production (capture & culture) during 1950-2004. (*Source: http://www.fao.org/fi/statist/statist.asp)*

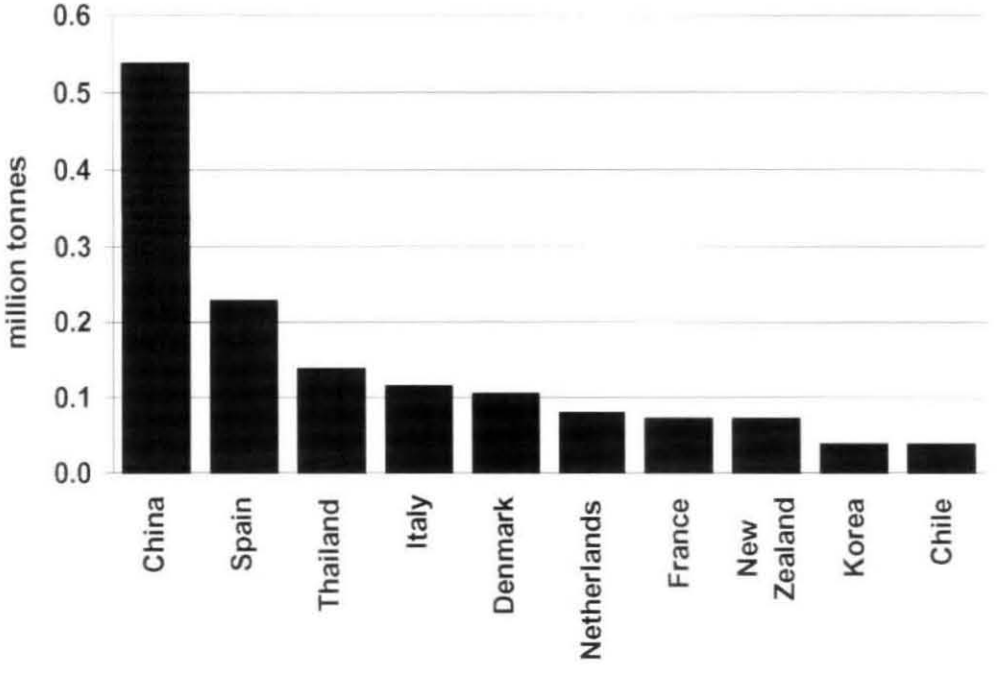

Fig. 1.2. Major mussel producing (capture & culture) countries of the world (1994-2004 average) (*Source: http://www.fao.org/fi/statist/statist.asp)*

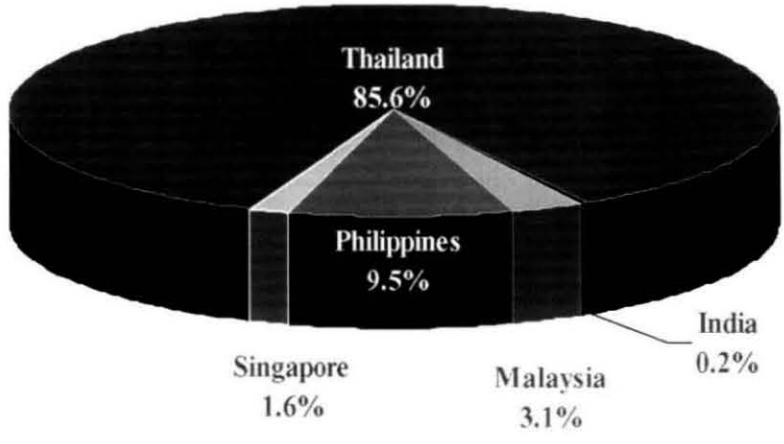

Fig. 1.3. Major green mussel producing (capture & culture) countries of the world (1994-2004 average) (*Source:http://www.fao.org/fi/statist/statist.asp)*

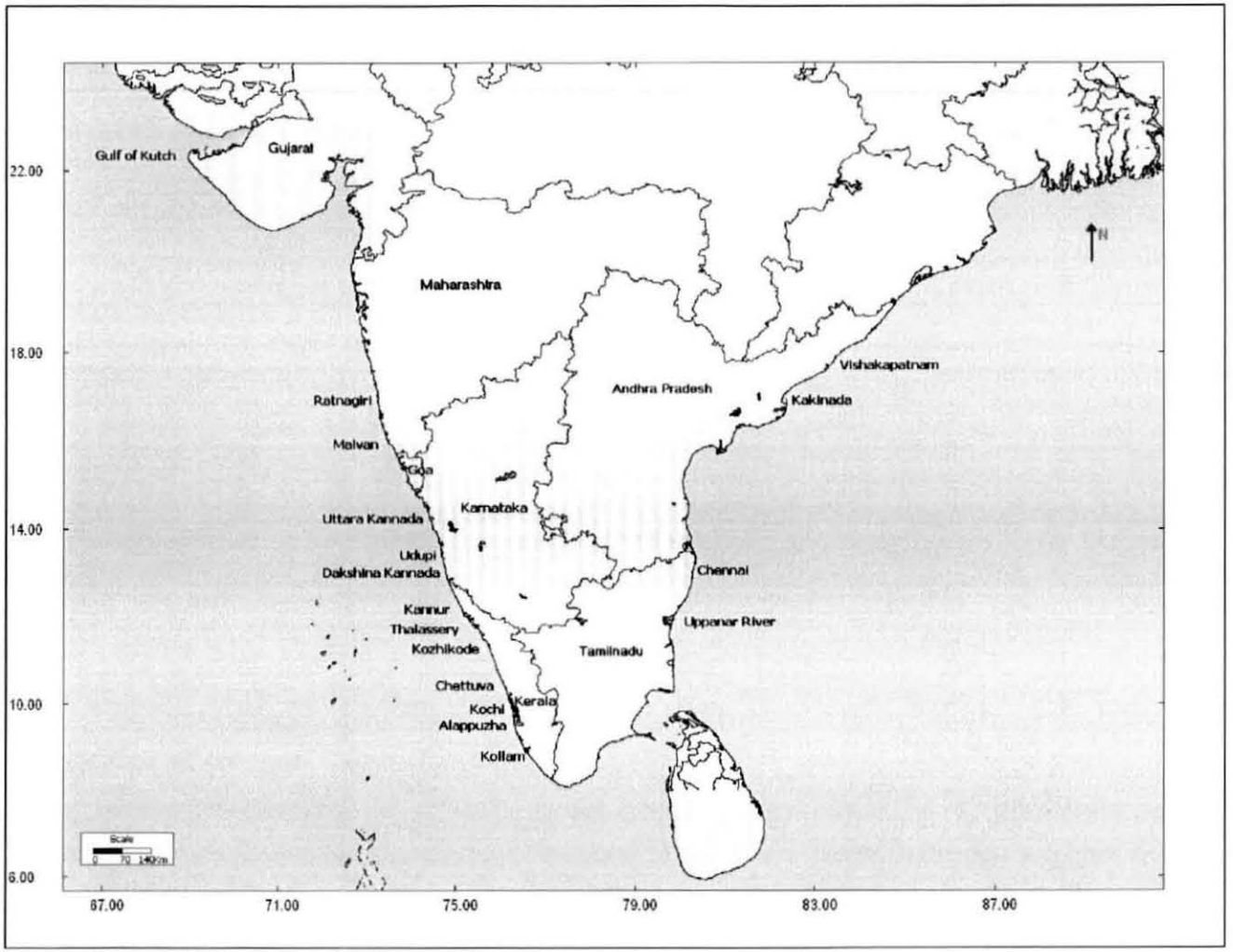

Fig. 1.4. Map showing the distribution of *Perna viridis* along the Indian coast

In Karnataka, rich natural beds of green-mussel, *P. viridis* exist in the intertidal and subtidal rocky areas along the coast. Mussel production of the state was estimated at 5,324 t during 2003-04 (CMFRI Annual Report, 2004; 2005). Among the three maritime districts of the State, mussel beds of Dakshina Kannada District contributed 36% (1,905 t) to the mussel production of Karnataka. In Dakshina Kannada dense settlements of green mussels are seen in the littoral and sub-littoral rocky stretches off Someshwara and Surathkal up to a depth of 6-10 m from the shoreline. These beds contributed 774 and 1,131 t respectively to the annual (2003-04) mussel production of Karnataka. Mussel beds in Dakshina Kannada are mostly subtidal in nature (Appukuttan *et al.*, 2001) and therefore, peak mussel fishing activity is observed from October to May after the monsoon. Mussel fishing is carried out by handpicking during low tides. Marketable size of green mussels generally range from 50 to 110 mm and is attained over a period of 5 to 18 months. Besides the traditional fishery from natural mussel beds, the area offers potential for mussel mariculture in coastal and estuarine waters of the coast as demonstrated by CMFRI (Mohamed *et al.*, 1998).

In Karnataka, there is scope for increasing the mussel production from natural resources by controlled and extensive exploitation. Further addition to the production can be attained by wider adoption of culture practices. However, in the present conditions, increased exploitation or any leap in production through farming could turn successful and economically viable only if backed up by appropriate marketing strategies. In addition, unlike other aquaculture species, the specific characteristics of bivalves and the bivalve ecosystem warrants attention while planning aquaculture developments.

Mussels, being sedentary in nature and through their filter feeding mechanism can accumulate a wide variety of substances including pollutants (chemical or biological) from the surrounding waters. Many of these pollutants act as stressors affecting the physiology of the bivalves and at higher concentrations it can lead to loss of the beds. Water quality of the mussel beds is influenced by freshwater inputs, sewage and effluent discharges, runoff from urban and agricultural land, offshore activities within the area and aerial deposition of pollutants. Each of these presents a route by which the environmental quality of a mussel growing area might become compromised. Therefore, an early detection of water quality problems is critical in preventing the deterioration and loss of mussel beds due to pollution. Assessment of the status of mussel growing areas to identify areas threatened by pollution and initiation of remedial actions to protect and restore water quality in the affected areas are important in this regard.

Further, when exposed to chemical pollutants or pathogenic microbial organisms even in very low concentrations, mussels can potentially accumulate such harmful substances to lethal concentrations. Pollutants accumulated by the bivalves are retained in their soft tissues, posing health hazard to the consumers.

Major substances of public health significance that could be accumulated by mussels are chemical pollutants such as trace metals, pesticides, hydrocarbons; pathogenic microorganisms and various biotoxins produced by marine organisms. Therefore, bivalve shellfish receives special consideration in food safety law throughout the world because they filter feed and become naturally contaminated in an unhealthy environment. Subsequent to the incidence of health problems associated with shellfish consumption and detection of contaminants in the products, strict quality control measures are implemented by different countries and international bodies controlling the seafood trade. These standards pertain to the quality of final product as well as the quality of the shellfish growing/ harvesting waters. In most of the developed countries, mussels can be marketed only if originating from clean waters, where regular analysis ensures the quality of the environment.

Several countries have developed Shellfish Sanitation Programmes in response to many disease outbreaks owing to the consumption of contaminated shellfish. The Canadian Shellfish Sanitation Programme (CSSP) and the National Shellfish Sanitation Programme (NSSP) of the USA were developed as early as 1925. The NSSP Guide for the Control of Molluscan Shellfish sets forth the principles and requirements for the sanitary control of shellfish produced and shipped in interstate commerce in the United States. It provides the basis used by the Federal Food and Drug Administration. Similarly, requirements for the sanitary control and hygienic production of live bivalve molluscan shellfish in European Union are covered by Council Directive 91/492/EEC (EC, 1991). In Australia, the Australian Shellfish Quality Assurance Programme (ASQAP, 2004), modelled on the US NSSP is used for classifying the shellfish growing areas of Australian waters. Similarly, New Zealand Shellfish Quality Assurance Programme (NZSQAP) classifies all commercial shellfish growing areas in New Zealand based on the requirements of US NSSP.

In India, though isolated incidents of (fatalities) shellfish poisoning were reported from states of Karnataka and Kerala (Karunasagar, *et al.*, 1984; 1998), so far such strict quality control measures are not mandatory for marketing shellfish in domestic markets. Shellfish quality control guidelines presently in force are mainly applicable to exports since it is made mandatory by the importing countries. Recent developments in the sector are indicative of sizeable increase in production while the concerns about coastal water pollution from various sources are mounting high.

Dakshina Kannada District is one of the most industrialized areas of the State wherein, more than five major industries are functioning. Many of these industries discharge their treated effluents into the sea at locations between the natural mussel beds off Someshwara and Surathkal, where most of the mussel fishing activity is concentrated. As a pollution control measure, the levels of specific contaminants in the coastal waters of Dakshina Kannada are monitored by various agencies to ensure compliance with the environmental quality

standards. Though such monitoring programmes provide valuable information regarding the levels of specific contaminants in the environment, it is not comprehensive enough to evaluate their effect on the bivalves. Moreover, the information available with reference to the presence of various contaminants which have a potential for bioaccumulation in bivalve fauna along the coastal areas under reference is inadequate to ascertain the possibilities of any health hazards associated with the consumption of shellfish harvested from the region.

Therefore, it is imperative to develop protocols and establish a system of continuous monitoring for the protection of the mussel beds and for the approval of harvests. Many countries have established frameworks for coordinated planning to protect and restore quality of shellfish growing waters. The shellfish improvement programmes are established to enable the shellfish waters to conform to the standards for a number of physical, chemical and bacteriological parameters. Development of a comprehensive plan primarily involves approval of mussel beds and potential culture sites based on the analysis of important environmental parameters and pollution threats of the area. Extensive study of physical, chemical and biological parameters of mussel beds, their temporal variations and influence on the biology and health of the mussels are important prerequisites for planning. Shellfish sanitation also becomes an important phase of the shellfish industry, the primary objective of which is to protect the public from the consumption of contaminated shellfish. Therefore, in many countries commercial harvest of shellfish is permitted only from approved production areas, which are regularly monitored. Developments in the sector can sustain only if the safety aspects are properly identified and addressed and an appropriate quality assurance and control system is put into operation.

A perusal of literature revealed that information on the physical, chemical and biological aspects of the mussel beds and health conditions of the mussels of the area are very limited. The green mussel is used in eco-toxicological studies for the evaluation of coastal waters along the Karnataka coast due to its wide distribution. These studies provide the baseline information on tissue levels of trace metals in mussels of the area (Krishnakumar *et al.,* 1990a,b, 1998). However, information regarding the levels of many contaminants such as persistent organic pollutants in the shellfish waters is wanting. Studies pertaining to the extent of sewage pollution of the mussel beds and the sanitary quality of mussels harvested from these mussel beds are limited to the work on the pathogens in coastal waters as well as bivalves of the nearby estuarine areas of the Dakshina Kannada District.

The present investigation entitled **"Studies on the coastal water quality in relation to the health of green mussel, *Perna viridis* (Linnaeus)"** was approached with an objective to analyse the environmental variables of mussel beds with emphasis on bio-effect monitoring and is envisaged to supplement the baseline information on the mussel beds off Someshwara and Surathkal of Dakshina Kannada District, Karnataka.

Biological-effect monitoring was attempted by monitoring the health of the mussel using non-specific biomarker, condition index, which integrates physiological responses of the mussels to multiple stressors. Variation in condition index was evaluated by monitoring it together with the natural stress factors in the mussel beds for gauging the seasonal and spatial variability. Presence of selected contaminants in the ecosystem and their presence in the soft tissues of the green mussel were analysed to assess the quality of coastal waters. The cytological response in the mussels was also studied to evaluate the impact of the pollutants on the mussels. Sanitary standards of the mussel beds were also investigated to examine the extent of sewage pollution of the shellfish waters.

Specific objectives of the study are:

- to study the temporal and spatial variations in selected physico-chemical parameters of the mussel beds along the Dakshina Kannada,
- to study the influence of selected environmental variables on the condition index (a non-specific biomarker of stress) of green mussels along the Dakshina Kannada coast,
- to study the concentrations of selected trace metals and organochlorine pesticides in the shellfish waters, their bioaccumulation in the soft tissue of green mussels and the biomarker response in the mussels, if any, along the Dakshina Kannada Coast and
- to study the extent of faecal contamination of mussel beds along the Dakshina Kannada Coast.

Chapter 2

Temporal variations in physico-chemical parameters of the mussel beds

2.1. Introduction

The green mussel *P. viridis* is typically found inhabiting the littoral to shallow sub-littoral zones of coastal waters, which are the most variable of all marine ecosystems (Newell, 1979). The physico-chemical parameters of the littoral and sub-littoral waters are influenced by tides, coastal currents, upwelling, precipitation, river inflows, land runoffs, domestic and industrial discharges and other anthropogenic interferences.

Being sedentary in habit, mussels are well adapted to harsh environmental conditions (Morton, 1987). It can tolerate short periods of exposure to extreme temperatures, salinities, desiccation and relatively high levels of turbidity due to suspended sediments. However, environmental factors such as temperature and salinity exert an overriding influence on the health of bivalves as they directly affect filtration rate, respiration and reproduction (Powell *et al.,* 1992, 1994; Hofmann *et al.*, 1994 and Kobayashi *et al.,* 1997). Further, inter-annual variations in temperature, rainfall and river runoffs exert a long-term influence on population productivity.

Physico-chemical parameters of coastal waters off southwest coast of India are highly variable due to the monsoon, upwelling and water currents. The southwest monsoons from June to September, characterised by strong southerly

currents along the west coast, induces upwelling of the subsurface water near the coast (Pillai *et al.*, 1997). Along the Dakshina Kannada coast, mussel beds are located sub-tidally (Appukuttan *et al.*, 2001) in the inshore areas and are exposed to considerable seasonal variations in physico-chemical parameters influencing their productivity.

Very few studies on the physico-chemical characteristics of mussel beds have been carried out along the Indian coast. While studying the growth of brown mussel, *Perna indica*, Appukuttan *et al.* (1980) noted annual variations in environmental features such as temperature and salinity off Vizhinjam coast. A reduction in temperature and salinity was reported during May-July with increased precipitation. Similar studies on the influence of various physico-chemical parameters on the growth, survival and reproduction of mussel are detailed by Kuriakose (1980) in Calicut, Narasimham (1980) in Kakinada and Parulekar *et al.* (1982) in Goa. Among the various factors, high salinity and rising temperature appeared to accelerate the growth of green mussel in Kakinada waters (Narasimham, 1980). Appukuttan *et al.* (1989) while studying the spat settlement of brown mussel *P. indica* in the southwest coast of India detailed the effect of temperature, salinity and dissolved oxygen content on spawning and settlement of spat on the natural mussel beds. Ajithakumar (1984) while investigating the reproductive physiology of *Perna viridis* and *P. indica* explained the ecophysiology of reproduction with the ecological conditions such as temperature, salinity, dissolved oxygen, turbidity and chlorophyll-a of mussel beds off Calicut and Vizhinjam. Along Karnataka coast, Thippeswamy (1990) detailed the environmental parameters while describing the population ecology of green mussel beds on the intertidal rocky shore off Someshwara coast.

In the present study, selected physical and chemical characteristics of the seawater of the mussel beds were analysed to study their spatial and temporal variations. Parameters considered in the study were temperature, rainfall, salinity, dissolved oxygen and pH.

2.2. *Review of literature*

2.2.1. *Physico-chemical parameters of coastal waters*

The physico-chemical parameters of the near-shore waters of Arabian Sea along the southwest coast of India have received the attention of several investigators (Qasim, 1978; Banse, 1988; Pant, 1992; Bhattathiri *et al.*, 1996; Gundersen *et al.*, 1998; Latasa and Bidigare, 1998; Sathyendranath *et al.*, 1999; Pillai *et al.*, 2000 and Gopinathan *et al.*, 2001). However, no attempt was made to study the variations in physico-chemical parameters of the mussel beds in specific, along Karnataka coast. Since investigations on the physico-chemical parameters of the mussel beds are scanty, a perusal of literature on the studies made on the physico-chemical parameters along the coastal waters of south Karnataka is attempted here for comparison.

Ramamirtham and Patil (1964) discussed the physico-chemical features existing along the shelf-waters off southwest coast including the prevailing water temperature, salinity and dissolved oxygen profile off Mangalore during the pre-monsoon period. Menon *et al.* (1977) studied the physico-chemical parameters of the inshore coastal waters off Mangalore and reported decrease in surface water temperature during the monsoon and early post-monsoon period. Suresh *et al.* (1978) described seasonal water temperature, salinity, dissolved oxygen and current patterns in the near-shore waters of Arabian Sea off Mangalore.

Benakappa *et al.* (1980) and Manjappa (1987) studied the physico-chemical parameters of Arabian Sea off Mangalore. Gupta *et al.* (1988a) recorded the monthly water temperature, salinity and dissolved oxygen levels of the inshore waters off Mangalore while studying the spatial and temporal variability in primary production. They also studied the physico-chemical parameters of the Arabian Sea while carrying out the ecological monitoring off Thaneerbhavi (Gupta *et al.*, 1988b). Ramesha (1989) explained the distribution of copepods with spatial and temporal variations in physico-chemical parameters in the coastal waters off Mangalore.

Rivonker and Verlecar (1990) studied the variations in temperature, salinity and dissolved oxygen off Mangalore. Channeshappa (1991) has recorded the changes in water temperature while correlating the oceanographic features with productivity of Arabian Sea off Mangalore. Lingadhal (1991) conducted ecological monitoring off Thaneerbhavi and reported the prevailing temperature, salinity, dissolved oxygen and pH levels of inshore waters. Lingadhal (1995) estimated the primary production along with the physico-chemical parameters in the Arabian Sea off Mangalore. Joseph *et al.* (1998) reported seasonal trends of the environmental parameters in the coastal waters off Mangalore such as temperature, salinity, dissolved oxygen, BOD, pH, nutrients, suspended load and chlorophyll-a. Low dissolved oxygen values of bottom waters with

corresponding high inorganic phosphates, indicative of monsoonal upwelling along the Mangalore coast was observed during September.

Pillai *et al.* (2000) detailed the influence of physico-chemical parameters on primary production of the eastern Arabian Sea. Environmental parameters of inshore waters of Arabian Sea were recorded by Lakshmipathi (2001) while studying the distribution of benthos in relation to sediment characteristics off Mangalore. Mendon *et al.* (2002) while investigating the distribution and abundance of zooplankton studied the environmental conditions off Mangalore. Shankar *et al.* (2005) reported the hydrography of the eastern Arabian Sea during monsoon. Krishnakumar and Bhat (2007) analysed the seasonal and interannual variations in oceanographic conditions off Mangalore coast for the period 1994 to 2004 and related the influence of these variations on the pelagic fishery.

2.2.2. Rainfall

Monsoon plays a significant role in the physical and chemical properties of the seawater. Changes in the physico-chemical properties of seawater are more pronounced in shallow littoral and sub-littoral zones since huge volume of water is drained into the zone through land runoffs. Southwest coast of India receives bulk of rainfall during the southwest monsoon (June-September) and the remaining fraction during northeast monsoon (October-November). With the onset of monsoon, upwelling occurs along the west coast of India due to the strong monsoon winds (Ramamirtham and Rao, 1974). Role of monsoon in influencing the productivity of the southwest coast of India has been discussed by several investigators (Subrahmanyan, 1969; Rajagopalan *et al.*, 1992 and Selvaraj *et al.*, 2003). Monsoon also plays a significant role in the ecology of the marine environment. Several commercially important fish and shellfish are known to have their peak spawning during the southwest monsoon months on the west coast of India (Ajithakumar, 1984 and James, 1992). The sudden variations of environmental factors of the ecosystem caused by monsoon trigger the reproductive behaviour of many species.

The temporal variations in the environmental parameters of mussel beds off the southwest coast are reported to be associated with the prevailing monsoon regime (Ajithakumar, 1984). In mussel beds, the important water quality parameters that might have a direct bearing on growth, survival and reproduction which are affected by rainfall are salinity, temperature, primary production and suspended particle load. In green mussel, peak spawning along the Kerala coast is reported during the southwest monsoon season (Alagarswami, 1980). Appukuttan *et al.* (1989) reported that with the onset of southwest monsoon, the salinity and temperature declines and this coincides with the commencement of spawning in natural beds of *P. indica* along Vizhinjam coast.

2.2.3. *Water temperature*

Water temperature is one of the most critical parameters of the marine ecosystem that influence distribution, abundance and growth of marine organisms. Besides the direct influence of temperature on metabolic activities of organisms, the physico-chemical parameters of the surrounding environment are largely affected by variations in temperature. Water temperature of an area is influenced by air temperature, wind speed, humidity, insulation, rainfall and water inflows.

Temperature has been widely recognized as an important factor in controlling growth rate of mussels. Chatterji *et al.* (1984) demonstrated that growth of *P. virdis* was significantly affected by temperature. Temperature is recognized as one of the major environmental determinants of metabolic rate and the level of activity of poikilothermic organisms. According to Sivalingam (1977) green mussel has a 50% survival temperature tolerance at 10°C and 35°C for periods of 2 weeks. However, its optimum temperature for normal growth was between 26°C and 32°C. Mussels like many other littoral invertebrates apparently unable to regulate their rate of heat loss or gain from the environment, are able to vary their rate of respiration and feeding rates in such a way as to maintain them relatively independent of the environmental temperature (Bayne *et al.*, 1976).

Though, local factors such as salinity, suspended particles and availability of suitable substratum can influence the local abundance of marine bivalves, their overall distribution is controlled by water temperature (Rylander *et al.*, 1996 and Segnini *et al.*, 1998). Most species of bivalves have a wide range of temperature tolerance but a narrow range over which they breed successfully. Thus water temperature is widely recognized as an important exogenous factor influencing reproduction and geographical distribution of the species (Giese, 1959).

The distribution of mussel populations in intertidal sites is determined by physiological intolerance of mussels to temperature extremes and desiccation. High water temperatures usually interact synergistically with desiccation to control upper zonal limits. Occasional, sudden and massive mortality at the upper limit of intertidal mussel bands are often correlated with prolonged periods of unusually high temperatures and associated desiccation stress (Suchanek, 1978, 1985 and Tsuchiya, 1983). Along the Indian coast, *P. viridis* is exposed to thermally stable hotter environment (Shafee, 1979).

Many workers have recorded the prevailing temperature trends along the coastal waters of Karnataka while studying the hydrographic parameters (Suresh, 1978; Channeshappa, 1991; Ramesha *et al.*, 1992; Thippeswamy, 1990; Gupta, 1998a,b; Manjappa, 1987 and Krishnakumar and Bhat, 2007). Similarly, Ajithakumar (1984) and Appukuttan *et al.* (1989) recorded the variations in temperature in natural mussel beds along the southwest coast of India. Thippeswamy (1990) discussed the changes in water temperature while describing the population ecology of mussel beds on the intertidal rocky shore

off Someshwara coast. Rajagopal *et al.* (1998a) studied the temporal variations in temperature in Kalpakkam coastal waters while monitoring the population ecology of *P. viridis.*

2.2.4. Salinity

Spatial and temporal changes in salinity can affect the occurrence, distribution and well being of sedentary bivalves. Salinity variations in seawater are mainly caused by river-runoff, precipitation and evaporation. Compared to offshore areas, coastal areas experience larger salinity fluctuations as a result of river runoff and this can lead to hyposaline conditions in coastal mussel beds.

Abrupt salinity fluctuations impair the physiological processes of marine mussels by disrupting their osmotic balance. When challenged by the salinity fluctuations, mussels respond immediately by closing their shell and then undertake iso-osmotic intracellular regulation by adjusting the intracellular concentration of ions, amino acids and other small molecules to maintain cell volume (Hawkins and Bayne, 1992). Thus in hypo-saline situations, mussels can effectively isolate from low salinity by closing its valves and maintaining a relatively high osmotic concentration within the mantle fluid (Davenport, 1979 and Aunaas *et al.*, 1988).

The green mussel is reported to have tolerance for reduced salinities (Morton, 1987). However, extremely low salinity conditions have a detrimental effect on growth and can even be lethal to mussels (Villela, 1984 and Gruffydd *et al.*, 1984). Sundaram and Shafee (1989) while investigating the salinity tolerance of *P. viridis* indicated the lower limits as 16 ppt under laboratory conditions. They noted the closure of valves for withstanding hyposaline situation even up to 2 days. In fluctuating saline conditions, as feeding is suspended while valves remain closed, growth rate will inevitably be depressed. Salinity variations are also reported to influence the growth as a result of reduced metabolic efficiency (Masilamoni *et al.*, 1997). Tedengren and Kautsky (1986) also reported lower growth rate and smaller maximum size as a result of salinity variations. Spawning in mussels has been reported to be triggered by changes in salinity (Parulekar *et al.*, 1982).

Chatterji *et al.* (1984) related the high growth rate of green mussel to higher salinities, which probably reflects the abundance of preferred phytoplankton in the environment added to the beneficial effects of salinity close to the optimum. According to Sivalingam (1977), the green mussel has a 50% survival salinity tolerance at 24 ppt and 80 ppt for period of 2 weeks. It was observed that the species have a wide range of salinity tolerance. The salinity for normal growth was reported to be above 27 ppt.

Few authors have studied the fluctuations of salinity in mussel beds along the southwest coast of India. Appukuttan *et al.* (1980) while studying the growth of *Perna indica* in mussel beds off Vizhinjam coast reported an annual variation in

salinity from 31.5 to 36.31 ppt with peak levels in May and October. Reduction in salinity levels was observed during May-July with increased precipitation. Similar trends in salinity variations in natural mussel beds were reported by subsequent studies along the southwest coast (Ajithakumar, 1984 and Appukuttan *et al.*, 1989).

2.2.5. Dissolved Oxygen

Dissolved oxygen is one of the most important indicators of water quality and consistently high level of DO signifies a healthy ecosystem. Generally, dissolved oxygen level of 4-7 mg/l is desirable for most of the aquatic animals. Dissolved oxygen input to the water is from the atmosphere and as a product of photosynthesis. Levels of dissolved oxygen in the inshore waters vary depending on factors like time of the day, season, depth, wave action, water temperature, salinity, pollution, eutrophication and upwelling. Incidentally hypoxic conditions in the surrounding waters may arise due to pollution, eutrophication and upwelling.

Mytilids belong to the facultative anaerobes that can live either aerobically or anaerobically but prefer to use oxygen (respiration) when it is available, as it allows a much more economical use of ATP molecule (de Zwaan and Mathieu, 1992). Mytilids are also called euryoxic because they tolerate a wide range of oxygen concentrations and adults are reported to have high tolerance of anoxia (de Zwaan *et al.*, 1991) Since intertidal and shallow waters are characterized by large fluctuations in temperature, salinity and oxygen availability, mussels tightly closes their shell valves to tide over unfavourable conditions (Davenport, 1985), which can induce an anaerobic type of metabolism. Moreover during intertidal exposure there is no respiratory gas exchange due to poor morphological adaptations for aerial respiration and a poor open circulatory system makes oxygen storage and transportation to tissues a difficult task leading to anaerobic respiration.

DO levels in the seawater varies from region to region and earlier studies on the DO variations of mussel beds along the southwest coast by Ajithakumar (1984) reported minimum DO levels in March, while Appukuttan *et al.* (1989) observed minimum values in September.

2.2.6. pH

pH is the measure of acidic-alkaline property of water and is measured as the negative logarithm of hydrogen ion concentration. The carbon dioxide produced by respiration of animals and plants in water has the effect of lowering pH. Whereas, carbon dioxide and bicarbonate removed from the water by the photosynthetic processes of aquatic plants increase the pH. Generally near neutral to slightly alkaline pH is considered ideal for the aquatic organisms. Prolonged and considerable variations in pH can cause disorders in metabolism

and growth and mass mortalities are reported in the event of significant variations in pH. Further, the pH of water affects solubility of nutrients, minerals, trace metals etc. Seawater is buffered against pH variations and hence under normal conditions the variations are minimal. A pH that is too high is undesirable because free ammonia increases with rising pH. The acceptable pH range for most finfish and shellfish species is 6.8 - 8.5.

Sivalingam (1977) observed that the green mussel has a 50% pH survival tolerance at pH 3.5 - 9 for periods of 2 weeks. It was also noted that the optimum pH for normal mussel growth is between 6.0 and 8.2. However, Bamber (1990) while studying the effects of acidic pH on three species of lamellibranch molluscs reported that pH < 7 are intolerable to bivalve molluscs. Significant mortalities were reported in *M. edulis* maintained for 30 days at pH < 6.6. Survival at a given pH level reduced with time of exposure and with increased temperature. Variations in salinity may cause changes in pH, but a fairly significant difference over a period of time is necessary to cause stress for bivalves (Quayle and Newkirk, 1989).

Seasonal variations in pH of Dakshina Kannada waters were reported earlier by few workers. Benakappa *et al.* (1980) reported the pH values in the fishing grounds off Dakshina Kannada and attributed the increase in pH to photosynthesis. Ramesha (1989) observed increase in pH during the pre-monsoon months and a reduction in October following the monsoon. pH fluctuations in the region was also attributed to the fluctuations in salinity and temperature in the near-shore stations (Segar, 1982 and Lingadhal, 1995).

2.3. *Materials and methods*

2.3.1. *Description of the Study area*

The study was conducted in the state of Karnataka along the southwest coast of India. The state has a coastline of 300 kilometres and is well known for its mussel beds. Among the three coastal Districts of the State *viz.*, Dakshina Kannada, Udupi and Uttara Kannada, Dakshina Kannada was selected for the present investigation.

Dakshina Kannada is naturally endowed with abundant freshwater resources as four rivers criss-cross the District and traverses the 42 km coastal stretch and form estuaries that are very productive. The Nethravati River (annual discharge 12,434 mcm) joins Gurpur River (annual discharge 2,915 mcm) forming the Nethravati-Gurpur estuary in the southern part of the District. Pavanje River (annual discharge 619 mcm) confluence with Sambhavi River (annual discharge 1,253 mcm) forming the Mulki estuary in the northern part of the District. Estimated average annual flow of the four rivers of the District is 17,221 mcm per year. The rivers are mostly rain-fed and the flow is closely related to the seasonal rainfall. Dakshina Kannada District gets an average annual rainfall of 4,000 mm every year. Southwest monsoon lashes the coast during June-September and during this period the rivers frequently overflow, while during the dry months (December to May) the rivers often experience periods of reduced flow.

Green mussel beds along the Dakshina Kannada coast are located in subtidal rocky stretches off Someshwara south of Nethravati-Gurpur estuary from Uchila to Ullal and south of Mulki estuary from Surathkal to Mukka. These areas are known for a seasonal and subsistence level fishery of green mussels, locally called as *pachila*. The peak levels of exploitation are observed during the fair weather season (October to April) in the area.

Dakshina Kannada District was selected as the study area considering the study objectives. Among the three maritime Districts of the State, Dakshina Kannada District is having the highest population density according to the Census, 2001 and the inshore waters of the coast receive discharge from several industrial units that could pose a threat to the fragile coastal environment.

2.3.2. *Selection of sampling stations*

In the study area, dense settlements of green mussels are seen on the submerged rocky patches in the littoral and sub-littoral zones of the coastline. Two distinct mussel beds are present in the area, one off Someshwara (12.79^0N; 74.84^0E) and the other off Surathkal (13.00^0N; 74.79^0E) (Appukuttan *et al.*, 2001).

Considerable mussel fishing activity takes place at these two centres during the season.

Two sampling stations, one at Someshwara and the other at Surathkal were fixed using a portable GPS, for the study representing the two major mussel beds. Sampling stations were identified and fixed at locations along the coast where peak fishing activities take place. Thus, station I, off Someshwara was fixed at 5.5 km south of Nethravati-Gurpur estuary and the station II, off Surathkal was fixed at 18.0 km north of Nethravati-Gurpur estuary and 9.0 km south of Mulki estuary (Fig. 2.1). At each selected sampling station, three sampling points were fixed at equidistant interval of 200 m parallel to the coast line.

2.3.3. Sampling

Mussel samples were collected from the sampling stations on a monthly interval between January 2003 and April 2004. The samples were drawn from a depth of 3-4 m, in the morning hours between 7:00 and 9:00 am.

Water samples were drawn using reversing water sampler (Hydro-bios) operated from a canoe and mussel samples were collected by skin diving. Field measurements of mussels were recorded immediately after the collection and the samples were then transferred to clean polythene bottles and stored in ice box for transport to laboratory for analysis.

2.3.4. Determination of physico-chemical parameters

Rainfall: The precipitation data in millimeters recorded by the Indian Meteorological Department was used in the study. The monthly rainfall data of the Dakshina Kannada District was used for both the stations.

***Temperature*:** Surface water temperature was measured *in situ* using a standard mercury thermometer. Bottom water temperature was read using a reversing thermometer mounted on the sampler.

pH: pH was measured using a pH meter of ±0.01 precision. The instrument was calibrated using standard buffers before use.

Salinity: Salinity was measured using a conductivity meter with a precision of ±0.01 ppt.

Dissolved oxygen (DO): Winkler's method was followed for the measurement of DO in seawater. Sub-samples of surface and bottom water were drawn into 125 ml BOD bottles and DO was fixed on site and transported to the laboratory for final analysis. DO was measured in mg per litre.

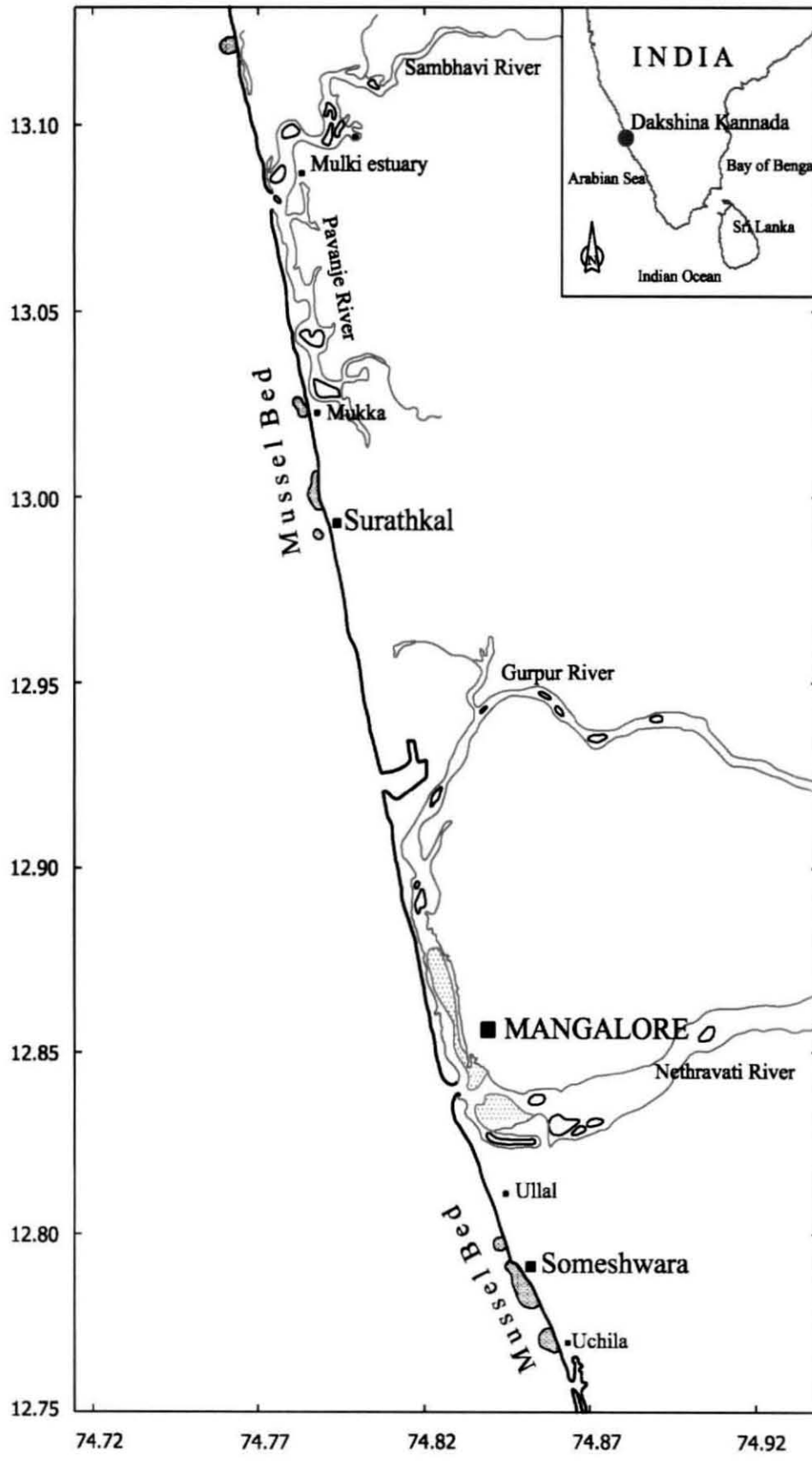

Fig. 2.1. Map showing the mussel beds along Dakshina Kannada coast (southwest coast of India), Karnataka

2.3.5. Statistical analysis

The physico-chemical parameters recorded from the three sampling points were averaged for each station for a given month. The seasonal and spatial variations in physico-chemical parameters of the mussel beds were analysed using one-way ANOVA. For comparing the seasonal variations monthly data were classified into three seasons *viz.*, pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January). Correlation (Pearson's) between the physico-chemical parameters and rainfall was carried out to understand the relationship among the parameters. Statistical analyses were carried out using SPSS (13.0) software.

2.4. Results

2.4.1. Rainfall

Seasonal variations in oceanographic features of Arabian Sea are primarily influenced by the prevailing monsoon regime. During the study period, southwest monsoon commenced by the last week of May and the highest rainfall (1,410 mm) was recorded in June (Fig. 2.2). Southwest monsoon weakened by the end of August. Moderate rainfall of 108-177 mm/month was recorded during October-November due to the northeast monsoon which is less prominent along the west coast. Monthly rainfall ranged from 6 mm in April to 1410 mm (40%) in June. Analysis of seasonal rainfall data (Table 2.1.) showed that the monsoon season accounted for 90% of the total rainfall (3,461 mm) followed by post-monsoon period.

2.4.2. Temperature

Someshwara: Monthly variations in seawater temperatures in mussel beds off Someshwara are presented in Table 2.2. Mean surface water temperature observed was 29.10±2.05°C. Highest surface water temperature recorded was 31.75°C in April and the lowest was 25.2°C in August. Mean bottom water temperature recorded was 28.59±1.85°C. Bottom water temperature measured at 27.90°C in January increased to a maximum of 31.15°C in April, then gradually declined to the lowest of 25.20°C in August and again increased to 29.0°C in October (Fig. 2.3). Analysis of variance indicated that there is no significant difference between surface and bottom water temperatures (Table 2.3). Seasonal variations in temperature was prominent with highest mean surface water temperature in pre-monsoon (30.86°C) followed by post-monsoon and monsoon seasons. Mean bottom water temperature during pre-monsoon was 30.12°C (Fig. 2.4). Analysis of variance showed that the fluctuations in water temperatures between the seasons were significant ($p<0.05$) (Tables 2.5, 2.6).

Surathkal: In mussel beds off Surathkal, the mean surface water temperature recorded was 29.36±1.69°C. It varied between 26.65 in August and 31.75°C in May (Table 2.2). The bottom water temperature ranged between 26.10 and 31.5°C with a mean of 28.85±1.76°C. The bottom water temperature measured at 27.30°C in January rose to a maximum of 31.45°C in May, then gradually declined to the lowest of 26.10°C in August and again rose to the second peak of 29.0°C in October (Fig. 2.3). Analysis of variance indicated that there is no significant difference between surface and bottom water temperatures (Table 2.4). Season-wise analysis of surface temperature in mussel beds off Surathkal indicated the highest mean values in pre-monsoon (31.11±0.72°C) followed by post-monsoon and monsoon seasons. Pre-monsoon bottom temperature was 30.76°C (Fig. 2.4). Analysis of variance showed significant difference ($p<0.05$) in water temperatures between the seasons (Tables 2.7, 2.8).

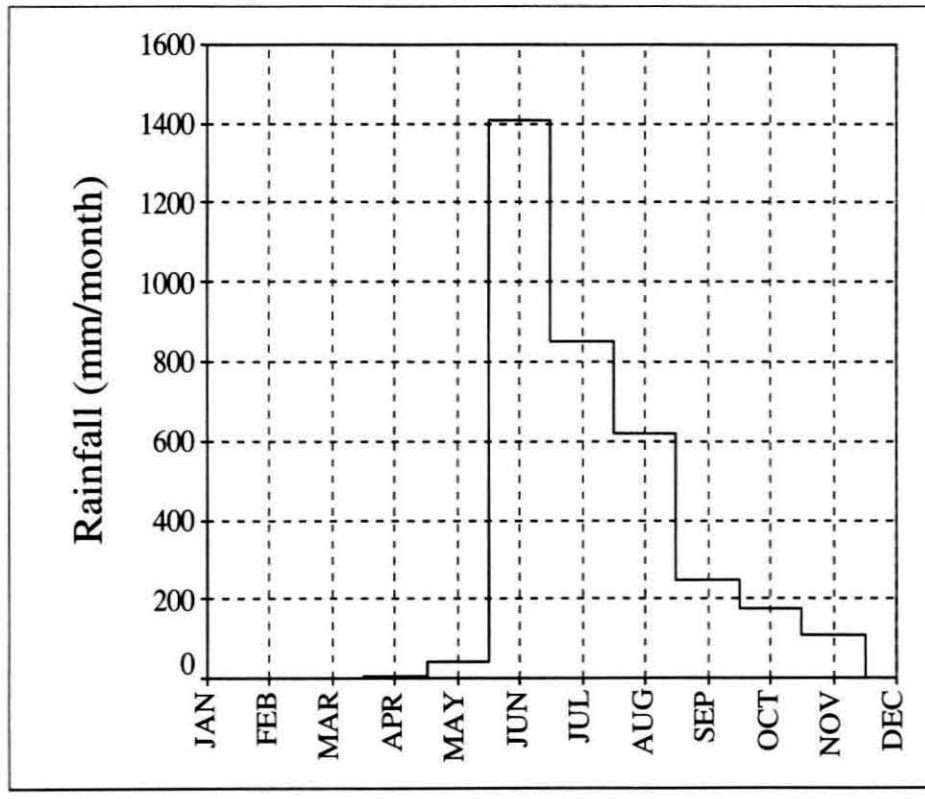

Fig. 2.2. Monthly variation in the rainfall pattern along Dakshina Kannada coast.

Table 2.1. Seasonal variation in the rainfall pattern along Dakshina Kannada coast.

Season	Total Rainfall (mm)
Pre-monsoon	45
Monsoon	3131
Post-monsoon	285

Analysis of variance indicated significant variation between the mussel beds off Someshwara and Surathkal with respect to water temperature in all seasons (Table 2.9).

2.4.3. Salinity

Someshwara: Wide variations in the salinity of coastal waters were noticed during the period (Fig. 2.3). The mean salinity of surface waters of mussel beds in Someshwara was 31.97±5.35 ppt. It varied between the minimum of 18.03 ppt in June and maximum of 35.38 ppt in May. Mean bottom salinity was 31.98±5.43 ppt and it varied from 17.53 to 35.42 ppt (Table 2.2). Analysis of variance indicated that there is no significant variation between the salinity of surface and bottom waters (Table 2.3). Season-wise analysis indicated that highest mean salinity of surface waters was 34.91 ppt during pre-monsoon followed by post-monsoon and monsoon (Fig. 2.4). Bottom water salinity also showed similar variations with season. ANOVA indicated significant seasonal variation in water salinity (Tables 2.5, 2.6).

Surathkal: At Surathkal, the mean salinity of the surface waters was 31.91±4.06 ppt and it varied from 25.13 ppt in August to 35.57 ppt in May (Table 2.2). Mean salinity of the bottom waters was 32.04±4.01 ppt with lowest of 25.27 ppt in August and highest of 35.55 ppt during May (Fig. 2.3). Analysis of variance indicated that there is no significant variation in salinity between surface and bottom waters (Table 2.4). Season-wise analysis indicated highest mean salinity at surface waters of 34.49 ppt during pre-monsoon followed by post-monsoon and monsoon (Fig. 2.4). The highest bottom salinity recorded was 34.76 ppt and it followed the same seasonal pattern of surface waters. Analysis of variance of salinity between the seasons showed significant variation ($p<0.05$) (Tables 2.7, 2.8).

Between the two mussel beds, salinity showed significant variation only during the monsoon season (Table 2.9).

2.4.4. Dissolved oxygen

Someshwara: Dissolved Oxygen (DO) levels of coastal waters were observed to be generally higher. At Someshwara, the mean surface water DO was 6.38±0.64 mg/l (Table 2.2). The lowest Oxygen content measured was 4.98 mg/l in October and the highest was 7.28 mg/l in July. The mean DO of bottom waters was 6.16±0.94 mg/l. It ranged from 4.41 mg/l in October to 7.14 mg/l in July in bottom waters (Fig. 2.3). Analysis of variance (Table 2.3) indicated that there is no significant variation between the DO levels in the surface and bottom waters. Seasonal analysis indicated that the DO values of surface and bottom waters were highest, close to saturation during monsoon followed by pre-monsoon and post-monsoon (Fig. 2.4). The surface DO was 7.09 mg/l and the bottom DO was

7.02 mg/l during the monsoon season. Analysis of variance showed that the DO levels of seawater (Tables 2.5, 2.6) differed significantly ($p<0.05$) with seasons.

Surathkal: The mean DO level of surface waters at Surathkal was 6.68±0.64 mg/l (Table 2.2). Highest DO levels in surface waters was 6.98 mg/l in August and the lowest was 4.63 mg/l in April. Mean value of bottom waters was 6.45±0.81 mg/l and it varied between 4.97 mg/l in November and 7.0 mg/l in July. Analysis of variance (Table 2.4) indicated that there is no significant variation between the DO levels in the surface and bottom waters. Seasonal analysis indicated that the DO levels of surface waters were highest during monsoon followed by post-monsoon and pre-monsoon (Fig. 2.4). Highest bottom DO was also noticed during monsoon followed by pre-monsoon and post-monsoon. ANOVA indicated significant difference ($p<0.05$) in DO levels of seawater (Tables 2.7, 2.8) between seasons.

Based on DO levels, significant difference was found between the mussel beds off Someshwara and Surathkal during monsoon and post-monsoon seasons (Table 2.9).

2.4.5. pH

Someshwara: At Someshwara, the lowest pH recorded for surface waters was 7.66 in July and the highest was 8.20 in November with the mean value of 7.97±0.19 (Table 2.2). Mean pH of the bottom waters was 7.98±0.19 with the lowest value of 7.65 in July and the highest of 8.20 in November. No significant difference was found between the pH of surface and bottom waters at Someshwara (Table 2.3). Season-wise variations of pH are presented in Fig. 2.4. Surface and bottom waters showed lowest pH during monsoon, followed by post-monsoon and pre-monsoon seasons. Analysis of variance indicated significant variation ($p<0.05$) in pH levels of seawater (Tables 2.5, 2.6) between the seasons.

Surathkal: At Surathkal, the annual mean pH of surface waters observed was 7.99±0.16, with the highest value (8.29) recorded in October and the lowest (7.78) in July (Table 2.2). Mean pH of bottom waters was 8.00±0.17 with the highest (8.27) in October and the lowest (7.78) in July. No significant difference was found between the pH of surface and bottom waters (Table 2.4). Season-wise variations of pH are presented in Fig. 2.4. Surface water pH during pre-monsoon was 8.08 which dropped to 7.82 in monsoon and rose to 8.07 in post-monsoon season. pH of bottom waters was 8.11, 7.83 and 8.07 during pre-monsoon, monsoon and post-monsoon seasons respectively. Analysis of variance showed significant variation ($p<0.05$) in pH levels of seawater (Tables 2.7, 2.8) between the seasons.

pH values of the two mussel beds differed significantly only during the monsoon season (Table 2.9).

Table 2.2. Monthly variations in physico-chemical parameters of mussel beds along the Dakshina Kannada coast.

Someshwara					
Month	**Depth**	**Temperature (°C)**	**Salinity (ppt)**	**DO (mg/l)**	**pH**
Jan	**S**	28.75±0.27	34.53±0.48	6.32±0.38	8.07±0.01
	B	27.90±0.66	34.60±0.44	5.97±0.49	8.07±0.02
Feb	**S**	29.00±0.55	34.53±0.18	5.90±0.12	8.09±0.05
	B	28.15±0.93	34.61±0.13	5.91±0.16	8.10±0.05
Mar	**S**	31.56±0.35	34.94±0.34	6.38±0.66	8.12±0.01
	B	30.70±0.61	34.96±0.45	6.31±0.80	8.14±0.03
Apr	**S**	31.75±0.27	34.84±0.33	6.49±0.18	8.06±0.04
	B	31.15±0.71	34.94±0.33	6.26±0.83	8.05±0.02
May	**S**	30.75±0.27	35.38±0.04	6.12±0.32	8.03±0.01
	B	30.20±0.22	35.42±0.04	6.01±0.12	8.06±0.02
Jun	**S**	27.40±0.00	18.03±1.01	7.01±0.09	7.79±0.03
	B	27.40±0.00	17.53±0.25	7.00±0.03	7.83±0.05
Jul	**S**	27.05±0.93	21.63±2.67	7.28±0.16	7.65±0.22
	B	27.05±0.93	21.66±2.71	7.14±0.09	7.65±0.21
Aug	**S**	25.20±0.00	31.06±0.06	7.15±0.01	7.71±0.02
	B	25.20±0.00	30.93±0.06	7.07±0.08	7.74±0.01
Sep	**S**	25.50±0.00	30.96±0.06	6.70±0.11	7.67±0.00
	B	25.50±0.00	31.00±0.00	6.73±0.07	7.69±0.02
Oct	**S**	29.50±0.00	34.60±0.26	4.98±0.20	8.04±0.04
	B	29.00±0.00	34.66±0.23	4.41±1.06	8.02±0.07
Nov	**S**	29.00±0.00	34.10±0.00	6.59±0.11	8.20±0.00
	B	28.00±0.00	33.95±0.07	6.37±0.07	8.20±0.00
Dec	**S**	28.00±0.00	33.88±0.68	5.91±0.50	7.98±0.09
	B	27.80±0.00	33.91±0.71	5.05±1.44	8.03±0.02
Mean	**S**	29.10±2.05	31.97±5.35	6.38±0.64	7.97±0.19
	B	28.59±1.85	31.98±5.43	6.16±0.94	7.98±0.19
Surathkal					
Jan	**S**	28.50±0.00	35.23±0.06	6.31±0.08	7.96±0.03
	B	27.30±0.00	35.20±0.00	6.47±0.17	7.99±0.00
Feb	**S**	30.66±0.50	33.60±2.12	6.95±0.51	8.07±0.03
	B	30.40±0.00	34.26±0.90	6.86±0.40	8.09±0.01
Mar	**S**	30.80±0.00	34.43±0.06	6.27±0.08	8.17±0.03
	B	30.20±0.00	34.47±0.06	6.41±0.10	8.24±0.02
Apr	**S**	31.50±0.00	34.73±0.12	4.63±0.13	7.99±0.02
	B	31.00±0.00	34.80±0.00	5.13±0.06	8.02±0.01
May	**S**	31.75±0.82	35.56±0.08	6.56±0.25	8.08±0.02
	B	31.45±1.15	35.55±0.05	6.58±0.16	8.12±0.00
Jun	**S**	27.60±0.00	30.73±0.06	6.58±0.03	7.86±0.05
	B	27.60±0.00	30.73±0.06	6.52±0.02	7.89±0.01
Jul	**S**	27.58±0.35	26.07±2.74	6.97±0.12	7.78±0.17
	B	27.38±0.13	26.03±2.70	7.00±0.18	7.78±0.18
Aug	**S**	26.65±0.20	25.13±2.12	6.98±0.12	7.80±0.03
	B	26.10±0.55	25.26±1.90	6.86±0.23	7.80±0.04
Sep	**S**	28.00±0.00	28.00±0.00	6.93±0.01	7.88±0.01
	B	27.50±0.00	28.10±0.00	6.88±0.09	7.90±0.00
Oct	**S**	29.50±0.00	33.40±0.00	6.71±0.08	8.29±0.05
	B	29.00±0.00	33.46±0.06	6.68±0.08	8.27±0.01
Nov	**S**	29.75±0.27	34.16±0.15	6.94±0.75	8.00±0.12
	B	28.75±0.27	34.30±0.16	4.97±1.38	7.98±0.19
Dec	**S**	29.25±0.82	34.66±0.38	6.97±0.37	8.07±0.02
	B	28.50±1.10	34.68±0.35	6.68±0.46	8.07±0.04
Mean	**S**	29.36±1.69	31.91±4.06	6.68±0.64	7.99±0.16
	B	28.85±1.76	32.04±4.02	6.45±0.81	8.00±0.17

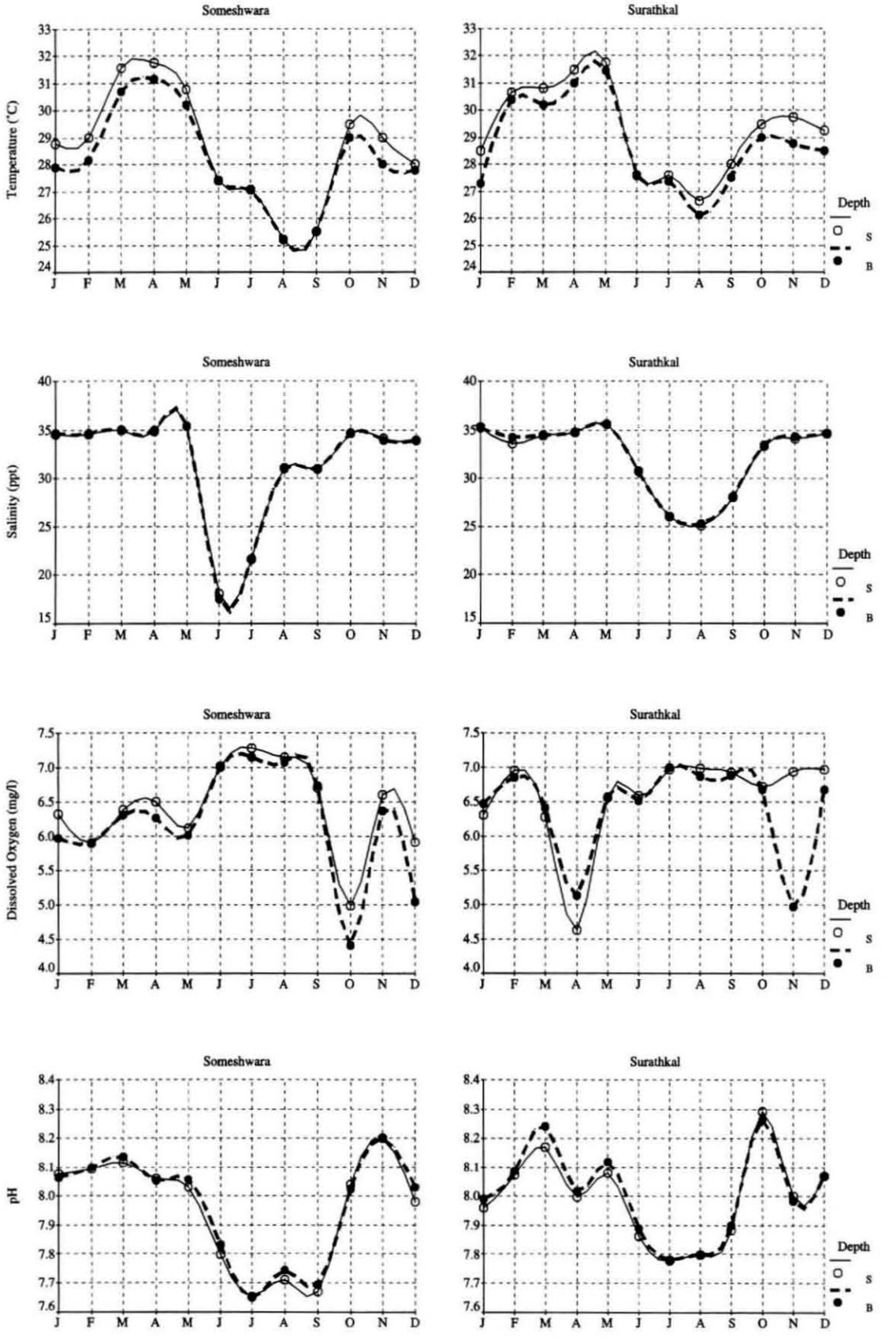

Fig. 2.3. Monthly mean surface (S) and bottom (B) seawater temperature, salinity, dissolved oxygen and pH off Someshwara and Surathkal mussel beds.

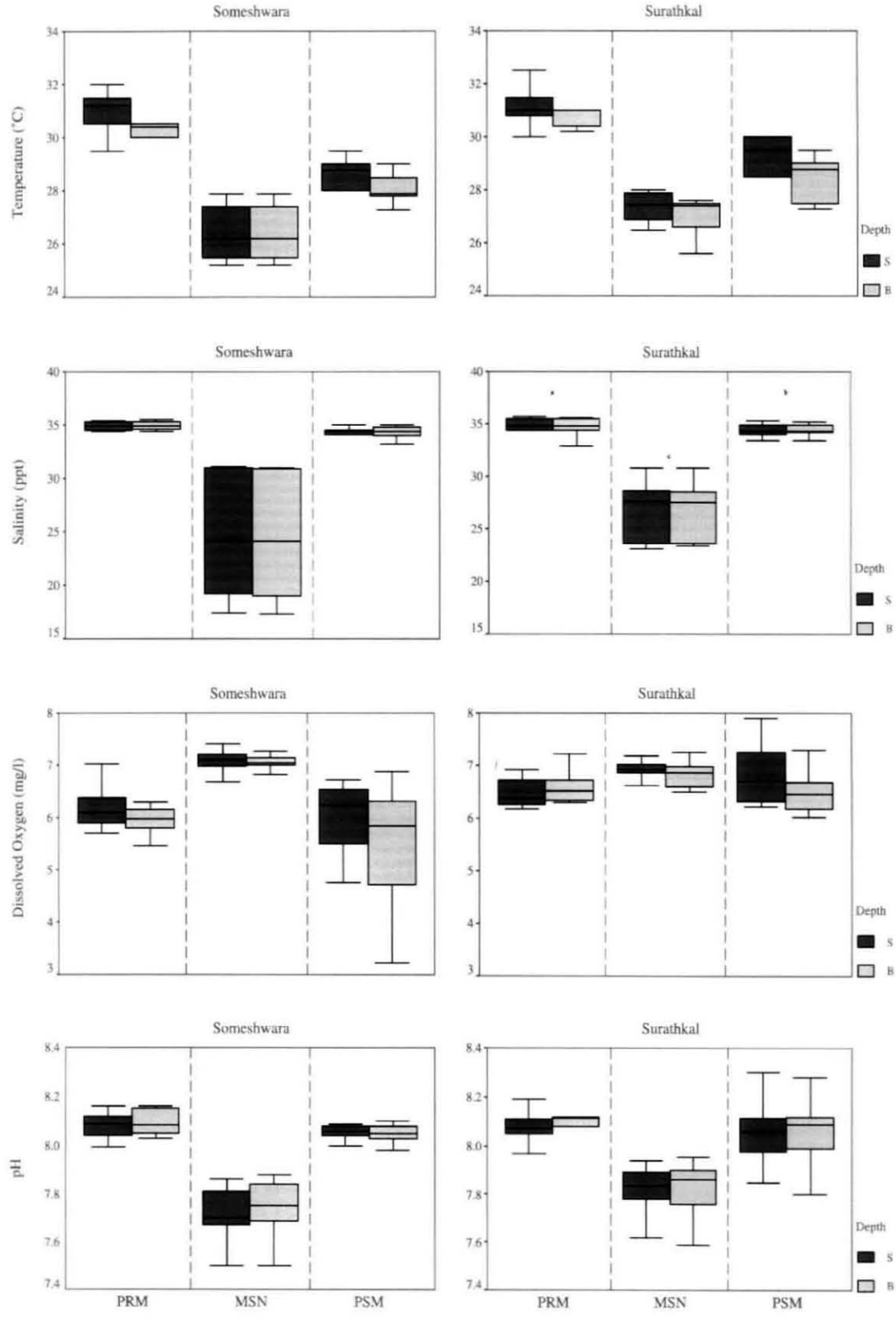

Fig. 2.4. The box and whisker summary plot representing (from top to bottom), the maximum, upper-quartile, median, lower-quartile and minimum values of surface (S) and bottom (B) seawater temperature, salinity, dissolved oxygen and pH off Someshwara and Surathkal during pre-monsoon (PRM), monsoon (MSN) and post-monsoon (PSM) seasons. The box represents the inter-quartile range which contains 50% of values.

Table 2.3. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of surface and bottom seawaters of the mussel bed off Someshwara.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Temperature x depth	Combined	7.8	1	7.80	2.044	*0.155*
	Within Groups	450	118	3.82		
	Total	458	119			
Salinity x depth	Combined	0.0	1	0.00	0.000	*0.996*
	Within Groups	3194	110	29.0		
	Total	3194	111			
DO x depth	Combined	1.5	1	1.45	2.248	*0.137*
	Within Groups	73.6	114	0.65		
	Total	75.1	115			
pH x depth	Combined	0.0	1	0.00	0.128	*0.721*
	Within Groups	3.9	110	0.04		
	Total	3.9	111			

Table 2.4. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of surface and bottom seawaters of the mussel bed off Surathkal.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Temperature x depth	Combined	7.4	1	7.43	2.500	*0.117*
	Within Groups	333	112	2.98		
	Total	340	113			
Salinity x depth	Combined	0.5	1	0.49	0.030	*0.862*
	Within Groups	1726	106	16.29		
	Total	1727	107			
DO x depth	Combined	1.5	1	1.46	2.748	*0.100*
	Within Groups	57.5	108	0.53		
	Total	59.0	109			
pH x depth	Combined	0.0	1	0.00	0.185	*0.668*
	Within Groups	2.8	102	0.03		
	Total	2.8	103			

Table 2.5. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of surface waters of the mussel bed off Someshwara in pre-monsoon, monsoon and post-monsoon seasons.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Temperature x Season	Combined	192	2	96	**99.1**	*0.000*
	Within Groups	55	57	1.0		
	Total	248	59			
Salinity x Season	Combined	1096	2	548	**61.1**	*0.000*
	Within Groups	475	53	9.0		
	Total	1572	55			
DO x Season	Combined	10.5	2	5.3	**22.7**	*0.000*
	Within Groups	12.7	55	0.2		
	Total	23.3	57			
pH x Season	Combined	1.5	2	0.8	**88.1**	*0.000*
	Within Groups	0.46	53	0.0		
	Total	1.98	55			

Table 2.6. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of bottom waters of the mussel bed off Someshwara in pre-monsoon, monsoon and post-monsoon seasons.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Temperature x Season	Combined	137	2	68	**60.5**	*0.000*
	Within Groups	64.7	57	1.1		
	Total	202	59			
Salinity x Season	Combined	1130	2	565	**61.0**	*0.000*
	Within Groups	490	53	9.3		
	Total	1621	55			
DO x Season	Combined	19.6	2	9.8	**17.5**	*0.000*
	Within Groups	30.7	55	0.6		
	Total	50.3	57			
pH x Season	Combined	1.48	2	0.7	**94.2**	*0.000*
	Within Groups	0.42	53	0.0		
	Total	1.90	55			

Table 2.7. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of surface waters of the mussel bed off Surathkal in pre-monsoon, monsoon and post-monsoon seasons.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Temperature x Season	Combined	137	2	68	162.6	0.000
	Within Groups	22.8	54	0.4		
	Total	160	56			
Salinity x Season	Combined	689	2	344	96.6	0.000
	Within Groups	182	51	3.6		
	Total	871	53			
DO x Season	Combined	3.16	2	1.6	4.4	0.017
	Within Groups	18.6	52	0.4		
	Total	21.8	54			
pH x Season	Combined	0.78	2	0.4	37.3	0.000
	Within Groups	0.51	49	0.0		
	Total	1.30	51			

Table 2.8. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of bottom waters of the mussel bed off Surathkal in pre-monsoon, monsoon and post-monsoon seasons.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Temperature x Season	Combined	139	2	69	115	*0.000*
	Within Groups	32.7	54	0.6		
	Total	172	56			
Salinity x Season	Combined	713	2	357	129	*0.000*
	Within Groups	140	51	2.8		
	Total	854	53			
DO x Season	Combined	5.5	2	2.7	4.7	*0.013*
	Within Groups	30.1	52	0.6		
	Total	35.6	54			
pH x Season	Combined	0.84	2	0.4	33.8	*0.000*
	Within Groups	0.61	49	0.0		
	Total	1.45	51			

Table 2.9. Analysis of variance of temperature, salinity, dissolved oxygen (DO) and pH of surface and bottom seawater between the mussel beds (station) off Someshwara and Surathkal.

Season	Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Pre-monsoon	Temperature x station	Combined	4.7	1.0	4.7	**4.2**	*0.044*
		Within Groups	107	94.0	1.1		
		Total	111	95.0			
	Salinity x station	Combined	2.2	1.0	2.2	2.9	*0.093*
		Within Groups	64.6	86.0	0.8		
		Total	66.8	87.0			
	DO x station	Combined	1.0	1.0	1.0	2.4	*0.124*
		Within Groups	34.5	86.0	0.4		
		Total	35.5	87.0			
	pH x station	Combined	0.0	1.0	0.0	0.5	*0.504*
		Within Groups	0.3	82.0	0.0		
		Total	0.3	83.0			
Monsoon	Temperature x station	Combined	8.9	1.0	8.9	**11.7**	*0.001*
		Within Groups	49	64.0	0.8		
		Total	58	65.0			
	Salinity x station	Combined	84	1.0	84.1	**4.5**	*0.038*
		Within Groups	1203	64.0	18.8		
		Total	1287	65.0			
	DO x station	Combined	0.5	1.0	0.5	**11.5**	*0.001*
		Within Groups	2.8	64.0	0.0		
		Total	3.3	65.0			
	pH x station	Combined	0.2	1.0	0.2	**14.2**	*0.000*
		Within Groups	1.0	64.0	0.0		
		Total	1.2	65.0			
Post-monsoon	Temperature x station	Combined	5.1	1.0	5.1	**9.0**	*0.004*
		Within Groups	39.7	70.0	0.6		
		Total	44.8	71.0			
	Salinity x station	Combined	0.3	1.0	0.3	0.7	*0.404*
		Within Groups	22.9	64.0	0.4		
		Total	23.2	65.0			
	DO x station	Combined	8.9	1.0	8.9	**9.9**	*0.002*
		Within Groups	63	70.0	0.9		
		Total	72	71.0			
	pH x station	Combined	0.0	1.0	0.0	0.2	*0.679*
		Within Groups	0.8	64.0	0.0		
		Total	0.8	65.0			

2.4.6. Relationship between the physico-chemical parameters of the mussel beds

Correlation coefficients between various physico-chemical parameters of mussel beds were analysed separately for the surface and bottom waters of the mussel beds. Both surface and bottom waters showed similar correlation pattern between different parameters.

Someshwara: In the mussel beds off Someshwara, salinity, temperature and pH of surface and bottom waters was found to have significant inverse relationship with rainfall (Table 2.10). DO was found to have significant positive relation with rainfall in surface and bottom waters as expected. Temperature was found to have significant positive correlation with salinity and pH and a negative relationship with rainfall. Salinity had a significant positive relation with temperature and pH of surface and bottom waters. Significant negative relationship of salinity was observed with DO only in surface waters. DO levels of surface and bottom waters were found to be positively correlated with rainfall and negatively related with salinity and pH. In surface and bottom waters pH had a significant positive relation with temperature and salinity. pH was negatively correlated with rainfall and DO in surface as well as bottom waters.

Surathkal: Correlation coefficients between various physico-chemical parameters of mussel beds off Someshwara are presented in Table 2.10. Correlation analysis indicated significant negative relationship of temperature, salinity and pH with rainfall. Temperature and salinity had significant positive relation among them and with pH in surface and bottom waters whereas, the rainfall had a significant negative influence. Temperature was also found to have a significant relation with DO levels of surface waters. Water pH was found to have a positive significant relation with temperature and salinity and negative significant relation with rainfall in surface and bottom waters.

Table 2.10. Correlation coefficient (Pearson's) between rainfall, seawater temperature, salinity, dissolved oxygen (DO) and pH of the mussel beds off Someshwara and Surathkal.

Someshwara						
Depth	**Parameters**	**Rainfall**	**Temperature**	**Salinity**	**DO**	**pH**
Surface	Rainfall	1.000	-.570(**)	-.949(**	.572(**)	.704(**)
	Temperature		1.000	.566(**)	-.330(*)	.767(**)
	Salinity			1.000	.581(**)	.753(**)
	DO				1.000	.493(**)
	pH					1.000
Bottom	Rainfall	1.000	-.492(**)	.953(**)	.468(**)	.710(**)
	Temperature		1.000	.495(**)	-0.168	.686(**)
	Salinity			1.000	.476(**)	.763(**)
	DO				1.000	.410(**)
	pH					1.000
Surathkal						
Surface	Rainfall	1.000	-.706(**)	.688(**)	0.176	.645(**)
	Temperature		1.000	.799(**)	.410(**)	.691(**)
	Salinity			1.000	-.299(*)	.784(**)
	DO				1.000	-0.21
	pH					1.000
Bottom	Rainfall	1.000	-.595(**)	.713(**)	0.234	.628(**)
	Temperature		1.000	.757(**)	-0.179	.647(**)
	Salinity			1.000	.365(**)	.776(**)
	DO				1.000	0.031
	pH					1.000

* $p<0.05$
** $p<0.01$

2.5. Discussion

Significant variability in the selected physico-chemical parameters was observed in both the mussel beds off Someshwara and Surathkal, on a temporal scale. Within the seasons, spatial trends were more evident during June-August. This variability was largely brought about by the changes in environmental factors associated with the monsoon regime. Variability of the selected parameters in the surface and bottom waters of the mussel bed was insignificant mainly due to the shallow nature of the mussel beds and vertical mixing as a result of tidal changes and wave actions.

During the period of study, southwest monsoon was active in Dakshina Kannada District from the last week of May to end of August with highest rainfall in June. Along the west coast, the monsoon starts during May-June in the south (Varkey, 2005) and progress northwards. Maximum rainfall along the coast is generally observed in June (Suresh, 1978; Ramesha, 1989 and Bhattacharya, 1991) with deviations (in July) in some years (Lingadhal, 1995 and Krishnakumar and Bhat, 2007). The total precipitation of 3,461 mm observed in the region was comparable with the normal annual rainfall reported in previous studies (Ramesha, 1989; Bhattacharya, 1991; Lakshmipathi, 2001 and Krishnakumar and Bhat, 2007). On analysing the seasonal trend in the region, it was found that the southwest monsoon contributes to nearly 90% of the annual precipitation. Ramesha (1989), Bhattacharya (1991) and Lakshmipathi (2001) also reported similar trends in rainfall with period of bulk precipitation during June-September. Monsoons have a profound influence on the variations in oceanographic features of Arabian Sea (Rao *et al.*, 1992). The rainfall was found to influence the environmental parameters of the mussel beds considerably. With the onset of monsoon a sharp decline in the water temperature, salinity and pH of the mussel beds was noticed. Rainfall was found to be negatively correlated with salinity, temperature and pH of surface as well as bottom waters. Similar influence of monsoon on the hydrographic features of the coastal waters of India has been discussed by many workers (Subrahmanyan, 1959; Rajagopalan *et al.*, 1992 and Pillai *et al.*, 2000). Large influx of fresh waters during the monsoon causes changes in most of the physico-chemical and biological parameters of the mussel beds which trigger many biological activities (Nagabhushanam and Mane, 1975 and Ajithakumar, 1984).

Water temperature followed seasonal variations with relatively high values in the pre-monsoon, followed by low values in the monsoon. The post-monsoon period was found to be a recovering period for temperature from the lowest recorded in August as a result of retreating rains. The sea surface and bottom temperature followed similar bimodal trends with a peak in pre-monsoon and another in post-monsoon. Similar bimodal trends in temperature variation have been observed in the coastal waters off Dakshina Kannada District by Suresh (1978), Channeshappa (1991), Ramesha *et al.* (1992) and Gupta *et al.* (1998a).

Variations in surface and bottom temperatures of mussel beds along the coast were insignificant due to the turbulence and wave action in the inshore waters. Mean temperatures of the two mussel beds varied significantly on a seasonal scale. This variation is mainly due to the variations in the runoff at the two stations.

The mean temperature values observed in the mussel beds registered a declining trend from May onwards coinciding with the onset of monsoon. However, the lowest temperature was reached only in August. Suresh *et al.* (1978), Manjappa (1987) and Krishnakumar and Bhat (2007) also observed lowest sea surface temperature along the Dakshina Kannada coast during August/September and attributed this reduction in sea water temperature to upwelling.

The mean bottom water temperature observed in the mussel beds off Someshwara and Surathkal ranged between 25.20 and 31.45°C during the period. The temperature range recorded from the mussel beds of the study area were comparable with earlier reports along the Indian coast (Ajithakumar, 1984; Appukuttan *et al.*, 1989 and Rajagopal *et al.,* 1998a) and within the temperature range reported for the normal growth of the species (Sivalingam, 1977). Lee (1986), Tan (1997), Masilamoni *et al.* (1997) and Benson *et al.* (2001) reported the occurrence of green mussels in coastal waters along its distributional range at temperatures between 10°C and 35°C. Further, the variation of 6°C between the maximum and minimum values observed in mussel bed in the present study occurred gradually over a period of three-four months, providing time for gradual acclimation.

Salinity of the mussel beds showed marked variations on a seasonal scale with low saline conditions prevailing during June-August (monsoon) which steadily increased from August reaching a maximum salinity of 35 ppt in May. Ajithakumar (1984) observed salinity values as high as 40 ppt in May in the mussel beds along the Kerala coast. The increase in salinity in pre-monsoon season corresponds to a decrease in river runoff and increase in temperature and evaporation. Similar trend in salinity was observed by earlier workers along the coastal waters of Dakshina Kannada District (Benakappa *et al.*, 1980; Rivonker and Verlecar, 1990; Thippeswamy, 1990 and Krishnakumar and Bhat, 2007). Between the two mussel beds of the study area, significant variation in salinity was observed only during the monsoon period which is attributed to relatively larger extent of dilution at Someshwara due to heavy river discharge from R. Nethravati.

In the mussel beds, even though the annual mean salinities were comparable, the rate of reduction in salinity was rapid with the onset of monsoon at Someshwara and the annual minimum levels were reached immediately by June. Whereas, at Surathkal the salinity reduction was gradual and the minimum levels were reached only in August. This is mainly due to the influence of freshwater influx from the Nethravati-Gurpur estuary during monsoon at Someshwara, where the

salinity variation too was comparatively higher due to the heavy river run-off. Lakshmipathi (2001) also recorded comparatively lower salinities off Mangalore section when compared to the Panambur (near Surathkal) section and attributed the condition to the influence of fresh water influx through the Nethravati-Gurpur estuary. During the present study, salinity of the mussel beds fluctuated between 17.7 ppt and 35.4 ppt. Annual fluctuations of 31.5 to 36.3 ppt in the mussel beds off South Kerala were reported by Appukuttan *et al.* (1980). Green mussels generally occur in coastal waters at high salinities between 27 and 35 ppt (Sivalingam, 1977; Lee, 1986; Tan, 1997; Masilamoni *et al.*, 1997 and Benson *et al.*, 2001) and under experimental conditions they were observed to tolerate low salinities up to 16 ppt (Sundaram and Shafee, 1989). In the present study the variations in salinity observed were within the tolerance limit of the species.

Dissolved oxygen level is one of the indicators of a healthy aquatic ecosystem. In the mussel beds, higher values of dissolved oxygen were observed during the monsoon season followed by a reduction in post-monsoon (Suresh *et al.*, 1978). The high level of dissolved oxygen during monsoon was consequent to a decrease in temperature and salinity, allowing higher solubility of gaseous oxygen in sea water as well as increased mixing associated with rough weather conditions. Similarly, the inverse relationship between temperature and dissolution rates (Gupta *et al.*, 1998a) accounted for the low DO level in pre-monsoon season. However, many investigators have attributed the reduction in oxygen levels during September/ October months to upwelling in inshore waters of Arabian Sea (Menon *et al.*, 1977; Suresh *et al.*, 1978; Rivonker and Verlecar, 1990 and Krishnakumar and Bhat, 2007). The mean DO levels observed in the mussel beds were comparatively higher because of the shallow nature of the sampling station where the intensity of wave action is relatively high (Lingadhal, 1991).

Dissolved Oxygen levels of the two mussel beds under study varied significantly during monsoon and post-monsoon seasons. This variability between the mussel beds might be due to the relatively low temperature and turbulence associated with high freshwater influx in Someshwara as compared to in Surathkal.

pH of mussel beds was found to be alkaline throughout the study ranging between 7.65 and 8.29. Krishnakumar and Bhat (2007) observed a pH of 7.24 to 8.58 off Mangalore. Seasonal analysis indicated a pH close to 8.0 in pre-monsoon; it dropped below 8.0 during monsoon and again increased in post-monsoon. Similar variations in pH were reported by Ramesha (1989); Rivonker and Verlecar (1990) and Thippeswamy (1990) in the coastal waters off Mangalore. Lingadhal (1995) and Lakshmipathi (2001) observed peak pH values during February, May and October/November in the Arabian Sea off Mangalore. Low pH was recorded in monsoon due to heavy run-off from rivers (Subramanian and Mahadevan, 1999) and high values in summer season due to higher levels of photosynthetic activity. Seawater is well buffered by its high

content of dissolved salts and more resistant to pH changes while heavy freshwater run-off from rivers which is less buffered decreases the pH of the inshore coastal waters (Yung *et al.* 2001). The pH was found inversely related to rainfall and directly related to the salinity and temperature. In general, the fluctuations in pH followed the variations in salinity and temperature (Lingadhal, 1995 and Segar, 1982). Lack of significant variations in the pH of surface and bottom waters indicated good vertical mixing in inshore waters. Comparing the mussel beds off Someshwara and Surathkal, significant variation in pH between the two beds was found only during the monsoon period. This is attributed to relatively higher degree of fresh water influx at Someshwara.

In summary, this chapter illustrates the variations in selected physico-chemical parameters of the mussel beds along the Dakshina Kannada coast of Karnataka. Mussels are capable of withstanding wide variations in salinity, desiccation, temperature and oxygen concentration, enabling them to occupy a large variety of microhabitats (Seed and Suchanek, 1992) and their distribution is principally influenced by seawater temperature (Seed, 1976 and Segnini *et al.*, 1998).

Study indicated significant variation between the mussel beds off Someshwara and Surathkal with respect to water temperature in pre-monsoon, monsoon and post-monsoon seasons whereas, salinity showed significant variation only during the monsoon season. Though the environmental parameters of the mussel beds presented spatial and temporal variations, the variability observed was within the limits for the optimal growth and survival of the species. The mussel beds off Someshwara and Surathkal being subtidal, the mussels were less exposed to extremes in temperature and associated desiccations. The variations in environmental parameters to a larger extent were found associated with the monsoon and spatial variability between the mussel beds were attributed to the differences in the volume of runoff diluting the inshore waters. Mussel beds off Someshwara presented wider variations in environmental parameters compared to the mussel beds off Surathkal.

The physico-chemical parameters detailed in this chapter were further investigated with reference to the health status of mussels and are presented in the following chapter (Chapter 3).

Chapter 3

Influence of environmental variables on the condition index of mussels

3.1. Introduction

Condition index (CI), expressed as the ratio of soft tissue weight to the shell weight, is one of the indices, generally regarded as an indicator of the health status of mussels. In marine monitoring studies, various physiological responses are used to provide "integrated" measures of an organism's well-being based on a range of different functional attributes. CI is employed in many studies involving marine invertebrates as an indicator of water quality as it integrates stress responses on somatic growth. As CI indicates the influence of environmental and contaminant stress on the physiological status of mussels (Lucas and Beninger, 1985), it has been projected for comparing contamination levels between different areas in "Mussel watch" monitoring programmes which employ tissue chemistry to evaluate the status and trends in environmental quality (Fisher, 1984; Martin *et al.*, 1984; Soto, 1995 and CIESM, 2002). In addition, CI also indicates the commercial quality of bivalve population and is used to follow seasonal changes in the gross nutrient reserves (Crosby and Gale, 1990).

Theoretically, in an unfavourable environment, an organism would use energy in response to the stress and therefore may have less energy available for growth. Typically in bivalves, the surplus metabolic energy after the requirements for maintenance and reproduction is converted into biomass under normal

conditions. Under adverse conditions, mussels that are stressed either by poor water quality or by disease have less energy for growth and less tissue reserves. The loss of tissue biomass during periods of negative scope of growth is the basis for the use of condition index as a measure of health in bivalves (Newell, 1985).

In bivalves, the soft tissue content follows seasonal variations associated with the reproductive cycles. The annual cycle of condition in mussel is described in terms of the extent to which the soft tissue fills the shell cavity, depending on the nutrient reserves and/or the amount of the reproductive tissue. Therefore, the variation in the glycogen content or reproductive tissue content is reflected in the relationship between the size of the bivalve and the meat content (Quayle and Newkirk, 1989).

The condition of mussel is largely controlled by the biotic and abiotic conditions of its environment. The exogenous factors that influence the temporal and spatial differences in accretion of somatic and/or gametes are reported as food availability, temperature and salinity (Seed and Suchanek, 1992). Any factor that result in altered food availability or the ability of mussels to assimilate this food, will alter the nutrient storage cycle (Newell *et al.*, 1982). Therefore, the concept that reduced health in mussels, brought about by limitations in food availability or other physiological stressors caused by unfavorable changes in environmental conditions can all result in poor conditions in bivalves (Bayne *et al.*, 1976).

Green mussels inhabit highly variable environments and are exposed to seasonal shifts in physico-chemical conditions such as temperature, salinity, dissolved oxygen as well as variations in food availability necessitating them to adopt a suite of physiological survival mechanisms. Condition index of bivalves is an easily measured physiological function used in environmental monitoring studies, as it integrates the physiological responses to stress on somatic growth (Nicholson, 1999). Such studies are generally adopted when comparisons are to be made between populations inhabiting different locations. Condition indices are used in such comparisons to ascertain whether the animals are in a relatively healthy or stressed state among the sites (Leavitt *et al.*, 1990). However, CI is not always indicative of stress when studied within a location, as they can be affected by seasonal changes and associated nutritional and reproductive stages.

In this study condition index and its seasonal and spatial changes were used to compare the populations and to study the influence of various environmental parameters on the well being of mussels. The data can also serve as baseline information for assessing the impacts of any future changes in the ecosystem. An attempt has been made to analyse the seasonal and spatial changes in condition index of mussels, the seasonal and spatial variations of selected environmental parameters of mussel beds and to relate the influence of these natural stressors on the condition index of green mussels for comparison of the general health status of mussel beds.

3.2. *Review of literature*

3.2.1. *Seston availability in shellfish waters*

Mussels are filter feeders feeding on a variety of suspended matter available in the mussel bed. Although the coastal waters where they inhabit are highly productive, the sessile nature of the mussels requires them to adapt to temporal changes in the seston (minute living organisms and particles of non-living matter which float in water and contribute to turbidity) and utilize what is available in their surrounding environment. The mussel beds are highly dynamic physical environments with temporal and spatial variations in the quantity and quality of available food (Widdows *et al.*, 1979; Rodhouse *et al.*, 1984; Smaal *et al.*, 1986; Bayne *et al.*, 1987 and Prins *et al.*, 1998). In such locations, advective process, resuspension and wave action can all affect temporal and large-scale spatial variability in food (Berg and Newell, 1986; Fréchette *et al.*, 1989; Prins *et al.*, 1996 and Smaal and Haas, 1997). Besides this, small-scale spatial variability is observed as a consequence of the filtration activity of bivalves (Fréchette and Bourget, 1985), depending on the degree of resuspension of seston (Smaal and Haas, 1997) and advection in the system (Prins *et al.*, 1996).

The amount of locally available food is dependent on the density of filter feeders, seston concentration and hydrodynamics (Dame and Prins, 1998). In dense aggregations of bivalves, the concentration of particles available for ingestion depends on the rate of particle replenishment by the currents. Natural beds of filter feeding bivalves are known to substantially deplete particle concentrations in the overlying waters during periods of low flow (Fréchette *et al.*, 1989). Recent simulation models of bivalve culture sites have brought together aspects of the dynamics of food supply (such as seasonal cycles of phytoplankton production and resuspension) with the physiological energetics of the cultured species in order to predict growth under field conditions (Grant *et al.*, 1993 and Dowd, 1997).

In marine environment, both composition and concentration of seston affect growth of mussels (Bayne *et al.*, 1989 and Smaal and van Stralen, 1990). Many studies have identified seston quantity and quality as the most important factors determining the energy budget of suspension feeding molluscs (Bayne and Newell, 1983; Bayne *et al.*, 1987; Navarro *et al.*, 1991; Hawkins and Bayne, 1992; Hawkins *et al.*, 1999). Hawkins and Bayne (1991) suggested that particulate organic carbon (POC) in the seston can play an important role in determining the overall energy budget of suspension feeding organisms. Bayne *et al.* (1993) observed varying rates of feeding and digestion in response to variability in seston concentration and organic content. Wind-induced resuspension has been reported to decrease the quality of the food available to bivalves while increasing the total quantity of suspended particles available whereas, increase in phytoplankton and POM increases the quality of the food

with higher organic particle loads (Berg and Newell, 1986 and Cranford *et al.*, 1998).

Among the suspended matter available in the environment, mussels ingest a variety of food like phytoplankton, detritus, bacteria and microzooplankton (Bayne and Newell, 1983). Among this, phytoplankton is the most important source of food of marine mussels (Grant, 1996). Hence, the concentration of phytoplankton and particulate organic matter in the water column primarily decides the availability of food to benthic suspension feeders. This study is therefore focused on the variations in quantity of seston in terms of phytoplankton (chl-a), suspended particulate matter (SPM), particulate organic matter (POM) and quality of seston (Chl-a/POM and POM/SPM) available to mussels in the mussel bed.

Phytoplankton concentration of coastal waters varies with region and season. Such variations in productivity could affect the dynamics of benthic suspension feeding communities because of the linkages between the food resources and the hydrographical changes occurring in the overlaying water column (Lesser *et al.*, 1994).

Quantification of phytoplankton production in water column includes estimation of total phytoplankton biomass or estimation of chlorophyll concentration. Chlorophyll-a (chl-a) has been used as a measure of phytoplankton standing stock since it serves as a useful indicator for both the photosynthetic potential and biomass of phytoplankton (Flemer, 1969). Its popularity in water monitoring programme is well known since it is a fairly accurate and simple measure of phytoplankton biomass (Dahlhoff and Menge, 1996).

The west coast of India with a wider continental shelf and more pronounced upwelling accounts to three-fourth of the primary production of the contiguous areas of the continental shelf of the sub-continent. Maximum productivity of the inshore waters of the west coast is observed during the upwelling season (Nair, 1974). Subrahmanyan (1959) made quantitative determination of the standing crop of phytoplankton of the west coast and found highest values during the south-west monsoon.

The phytoplankton distribution and primary production of the west coast have been illustrated by Nair (1974) and others. Qasim (1978) described the biological productivity of the coastal waters of Indian Ocean. Considerable information is available on the chl-a and primary productivity of Arabian Sea (Banse, 1987; 1988; Pant, 1992; Bhattathiri *et al.*, 1996; Gundersen *et al.*, 1998; Latasa and Bidigare, 1998; Sathyendranath, *et al.*, 1999; Pillai *et al.*, 2000 and Gopinathan *et al.*, 2001). Along the Dakshina Kannada coast distribution of chl-a is detailed by Rivonker and Verlecar (1990), Joseph *et al.* (1998), Manjappa (1997), Lingadhal *et al.*, (2003) and Krishnakumar and Bhat (2007).

Besides phytoplankton, mussels derive their nutrition from other particulate organic matter, the availability of which varies in the mussel bed due to the interaction of numerous biological and physical factors. Particulate organic matter (POM) is the organic matter that is retained on a 0.45 µm sieve and is composed of living and non-living (detritus) components. In seawater, the organic carbon in the form of dissolved organic matter/carbon or particulate organic matter/carbon (POM or POC) in the upper ocean is recycled, but a significant percentage of the carbon in the form of remains of organisms (POM) sinks to the seafloor. Upon reaching the seafloor the POM may be consumed by filter or deposit feeding organisms living in the bottom, or it may settle in the sediments.

In the aquatic system, particulate organic matter (POM) forms the organic portion of the Suspended Particulate Matter (SPM). Temporal variations in suspended particulate material can arise from freshwater inflows, rainfall regimes, upwelling, and eutrophication. Periods of high flooding in rivers associated with rainfall bring in higher concentration of suspended particulate material than those observed during low-flushing periods.

As a non-toxic stressor, suspended solids can reduce light penetration when in suspension, thus affecting primary production (Lloyd, 1987). Adverse effects on organisms can also occur due to mechanical abrasion of gills, reduction in feeding rates and increased susceptibility to diseases (Newcombe and Mac-Donald, 1991 and Leverone, 1995). Deposition of suspended solids can cause adverse effects by smothering benthic organisms and their habitats, which, in turn, reduce the food supply and refuge for many bottom dwelling animals (Hogg and Norris, 1991 and Rice and Hunter, 1992). Shin (2002) while investigating the lethal and sublethal effects of suspended solids on the survival, physiological, behavioural and morphological changes of the green-lipped mussel *Perna viridis* showed that it can survive in test conditions of suspended solids from 0 to 1200 mg/l over a period of 96 h in the laboratory. However, in natural environment bivalves are particularly vulnerable to the effects of elevated levels of suspended solids owing to their filtering mechanism. Studies on physiological responses of bivalves to increasing suspended sediment concentrations showed decrease in clearance rate (Bricelj and Malouf, 1984; Ward and MacDonald, 1996 and Bacon *et al.,* 1998), oxygen consumption (Grant and Thorpe, 1991) and growth (MacDonald *et al.,* 1998).

Suspended particles in the mussel beds were analysed for suspended particulate matter (SPM), particulate organic matter (POM) and particulate inorganic matter (PIM).

3.2.2. Condition index

The accretion of the somatic or gonadal tissue biomass has been extensively studied in mussels in connection with ecological consequences of the

interrelationships with their environment (Bayne and Hawkins, 1992); in sustenance fisheries; in mariculture (Hickman *et al.*, 1991; Camacho *et al.*, 1995; Reiriz *et al.*, 1996; Iglesias *et al.*, 1996 and Cheshuk *et al.*, 2003). Condition indices are used in bivalves for assessing the effect of long-term stress on bivalve quality (Maguire *et al.*, 1999) for measuring physiological responses in environmental monitoring (Krishnakumar *et al.*, 1994; Nicholson, 1999 and Shuhong *et al.*, 2005); in temperature tolerance studies (Sphigel *et al.*, 1992 and Isono *et al.*, 1998); in monitoring the variations in exposure duration (Montaudouin, 1996) and in response to parasitic infestation (Paynter and Buresson, 1991 and Chu and Volety, 1997).

The quantitative variation in somatic tissue and/or gametes in mussels are controlled by endogenous (genotype, body size and physiological status) and exogenous factors. The most important exogenous factors are food availability, temperature and salinity (Bayne and Newell, 1983 and Seed and Suchanek, 1992). The reproductive cycle in mussel is a genetically controlled response to the environment (Sastry, 1975). Mussels undergo annual reproductive cycles that are associated with marked seasonal changes in gross biochemical composition and physiological processes (Bayne and Newell, 1983; Hawkins and Bayne, 1992 and Rodhouse *et al.*, 1984). Although the reproductive cycle in mussels is species specific, the timing and duration are determined by the interaction between endogenous and exogenous factors, which are varying with the geographical locations and environmental conditions (Rodhouse *et al.*, 1984; Seed and Suchanek, 1992 and Villalba, 1995). The most important environmental parameters affecting the bivalve reproductive process are temperature and food availability (Lubet, 1981; Seed and Suchanek, 1992; Pazos *et al.*, 1997 and Ceballos *et al.*, 2000).

The role of endogenous factors in modulating individual responses will depend on the nature, amplitude and frequency of environmental change (Hawkins and Bayne, 1992). Direct responses and the survival vary according to potentially synergistic interaction between various environmental variables (Newell, 1979 and Bayne and Newell, 1983). Primary production was significantly correlated with CI in *M. edulis* (Smaal and van Stralen, 1990). Hickman *et al.* (1991) correlated reduced salinity with improved mussel condition index, higher nutrient levels, higher chl-a and larger quantities of particulates in *Perna canaliculus* in New Zealand.

Along the Indian coast, Narasimham (1980) recorded high CI in *P. viridis* from natural beds, during the resting phase starting from July to December in the east coast. The whole tissue dry weight in *P. viridis* from natural beds off Vizhinjam and Elathur along the west coast of India followed the reproductive cycles and the seasonal fluctuations in phytoplankton biomass (Ajithakumar, 1984). Rajagopal (1998a) observed peak gonadal index in *P. viridis* during April-May and September-October, with seasonal peaks in temperature and spawning activity. The percentage edibility or wet meat percentage of *P. viridis* monitored

in suspended culture by Parulekar *et al.* (1982), Rivonker *et al.* (1993) and Mohamed *et al.* (1998) along the Indian coast were related to variations in environmental parameters.

In mussel, apart from food availability, temperature and salinity also exert considerable influence on condition (Bayne and Newell, 1983; Seed and Suchanek, 1992). Among the various exogenous factors which influence reproduction in marine mussels, sea temperature has received most attention. Temperature has a direct effect upon the metabolic rate, seasonal patterns in growth and reproduction in bivalves. Seed (1976) indicated that temperature is a principal factor in controlling the broader aspects of the annual cycle of mussels. It is widely suggested that spawning in lamellibranches occurs only over a critical temperature range which is constant for each species. Bayne (1975) described a relationship between rate of gametogenesis and rate of change of temperature. Species are more likely, however, to have several critical spawning temperatures depending on their physiological condition or geographical distribution and adaptation to spawn at different temperatures especially at limits of distribution (Seed 1976). Seed and Suchanek (1992) have suggested that a 'temperature window' may exist outside which gametogenesis declines or does not occur, but inside which the reproductive strategy will depend to a large extent on the food availability. This window presumably varies according to the temperature range normally experienced by any particular population and to which it will therefore be adapted. Bayne and Worall (1980) showed that gamete production is initiated by a rise in temperature only if sufficient nutrients are available either as energy reserves or as recently ingested food. Increasing and/ or decreasing temperatures are reported to stimulate spawning in marine mussels (Kautsky, 1982 and Wilson, 1987).

Rajagopal (1998a,b) showed that breeding activity of the mussels along southeast coast of India was largely influenced by temporal distribution of seawater temperature. Mussels exhibit two spawning periods and temperature appears to regulate the onset of reproductive events (Rajagopal, 1998b). Chen *et al.* (1998), while studying the spawning period of *P. viridis* observed spawning in May-September when the temperature is 23-26°C. In tropical waters, *P.viridis* is exposed to thermally stable but hotter environment (Shafee, 1979). Even then, changes in temperature have been reported to affect growth rate (Chatterji *et al.*, 1984 and Vakily, 1992) in adult mussels and growth, survival and settlement in spat (Nair and Appukuttan, 2003).

Salinity changes in the external environment may disrupt the steady-state balance in mussels causing stress (Hawkins and Bayne, 1992) resulting in growth retardation (somatic and shell) (Parulekar *et al.*, 1982). Salinity also plays an important role in bivalve reproduction (Nagabhushanam and Mane, 1975). In the estuarine waters of Goa, the annual cycle of reproduction in *P. viridis* was related to the temporal variation in temperature and salinity (Parulekar *et al.*, 1982).

3.3. Materials and methods

3.3.1. Water sampling

Seawater samples were collected from Someshwara and Surathkal mussel beds as described in chapter 2, from three sampling points fixed at an equidistant interval of 200 m, parallel to the coast line at 3-4 m water depth. Samples were drawn from surface and bottom at the selected sampling points on a monthly interval using reversing bottle operated from a canoe. The samples drawn were transferred to sampling bottles and transported in insulated box to the laboratory for analysis.

3.3.2. Seston analysis

The chl-a, SPM and POM were used as indices for the quantitative analysis of seston available in the mussel beds. The ratios of POM/SPM and Chl-a/SPM were used for assessing seston quality.

3.3.2.1. Chlorophyll-a (chl-a)

Chl-a content of the water was measured by spectrophotometry after vacuum filtration and extraction in acetone. Two litres of seawater sample drawn from three sampling points (surface and bottom each) were collected for chlorophyll analysis. Sub-samples of 500 ml were filtered through Whatman GF/C filter paper. Magnesium carbonate was added during filtration to retard degradation and enhance filtration efficiency. The pigments were extracted overnight with 10 ml of 90% acetone in the dark at 4°C and extract was centrifuged and chl-a in the supernatant was determined by spectrophotometry (Strickland and Parsons, 1968).

3.3.2.2. Suspended Particulate Matter (SPM)

The SPM was determined by gravimetric method. A sub-sample of 500 ml of seawater was filtered through 47 mm GF/C pre-combusted (for 2 h at 450°C) and pre-weighed (±0.001 mg) filter paper to estimate SPM (Wong and Cheung (2001). Salt was removed from the filter by rinsing with distilled water and the filter paper was dried at 110°C and weighed. The SPM was estimated as the weight of filtrate retained, expressed as mg per litre.

3.3.2.3. Particulate Inorganic Matter (PIM)

The filters used for determination of SPM were again ashed in a muffled furnace at 450°C as detailed by Wong and Cheung (2001) and weighed. PIM was determined as the difference in weight of filters before and after combustion and expressed as mg per liter.

3.3.2.4. Particulate Organic Matter (POM)

The amount of POM suspended in water can be estimated by first removing the suspended material from the water by filtration, followed by either a direct measurement of the amount of carbon retained on the filter or by estimating the amount of carbon present from the weight lost upon heating the filter in excess of 450°C (Wong and Cheung, 2001). Organic seston (POM) concentration was estimated as the difference between SPM and PIM and expressed as mg per litre.

The seston quality indices, Chl-a/POM and POM/SPM were calculated from the values of Chl-a, SPM and POM.

3.3.3. Condition index

At least seven different condition index formulae are currently in use for bivalves (Davenport and Chen, 1987 and Crosby and Gale, 1990). In these methods of estimation, the shells are quantified by weight, volume or by the space it encloses whereas; the tissue is assessed variously on the fresh, dried or cooked conditions.

Crosby and Gale (1990) reviewed and evaluated bivalve CI methodologies and proposed methods to minimise errors. The first definable quantitative condition index equation which served as the outline for a multitude of modern condition index formulae is of A. E. Hopkins (Crosby and Gale, 1990). Accordingly the condition index is calculated as:

$$CI = \frac{\text{Dry soft tissue wt. (g) x 100}}{\text{Internal shell cavity volume (ml)}} \quad \text{----------(1)}$$

Where, the internal shell cavity volume is the difference between the total displacement volume of whole bivalve and shell volume.

Walne (1970) modified the index in an order of magnitude by altering the formula as follows:

$$CI_{vol} = \frac{\text{Dry soft tissue wt. (g) x 1000}}{\text{Internal shell cavity volume (ml)}} \quad \text{----------(2)}$$

Walne and Mann (1975) further modified this formula applying dry soft tissue weight and shell weights as presented in equation 3. This equation was later recommended by Lucas and Beninger (1985) for use in bivalve aquaculture.

$$CI_{shwt} = \frac{\text{Dry soft tissue wt. (g) x 100}}{\text{Dry shell weight (g)}} \quad \text{----------(3)}$$

Hickman and Illingworth (1980) and Lawrence and Scott (1982) revised the formula by changing dry shell weight with internal shell cavity capacity in weight as:

$$CI = \frac{\text{Dry soft tissue wt. (g) x 100}}{\text{Internal shell cavity capacity (g)}} \quad \text{----------(4)}$$

where, internal shell cavity capacity is the difference between the total bivalve weight and the dry shell weight.

Hawkins *et al.* (1987) calculated condition index with a modification of equation 4 as given below:

$$CI_{grav} = \frac{\text{Dry soft tissue wt. (g) x 1000}}{\text{Internal shell cavity capacity (g)}} \quad \text{----------(5)}$$

In general, methods utilizing wet flesh weight or wet flesh volume are less sensitive largely due to the difficulty in standardizing the degree of wetness.

According to Crosby and Gale (1990), the CI_{vol} and CI_{grav} techniques yield indices, which assess the proportion of available internal cavity capacity utilized by a bivalve's soft tissue. The CI_{shwt} technique is not a measure of how much available space is utilized and does not account for possible variations of internal cavity capacity due to overall shape and shell thickness variability in bivalves. Thus, it is not an index of nutritive status and therefore they have recommended CI_{grav} or CI_{vol} to ascertain the nutritive status of bivalves or to determine whether the animals are under stressful condition. However, they observed that for CI_{vol}, the coefficient of variation was ~25% greater than for CI_{grav} and recommended CI_{grav} due to low rate of measuring error, less variability surrounding the mean and the simplicity of technique.

Hickman and Illingworth (1980) has recommended the meat percentage, an index based on the wet meat weight or $CI_{commercial}$ for use in field and in mussel farming practice,

$$CI_{commercial}\ (\text{Meat yield}) = \frac{\text{Wet tissue wt. (g) x 100}}{\text{Whole (live) weight (g)}}$$

In the present study, CI_{grav} was used for calculating mussel condition index (CI) and $CI_{commercial}$ (meat yield) was used for studying the variations in wet meat percentage or the percentage edibility (% edibility).

3.3.4. Sample preparation and analysis

Condition index is measured either for individual mussels or for whole population. However, considering the variability, individual measurements are preferred whenever condition is investigated in relation to biological aspects (Seed and Suchanek, 1992). In the present study, the CI was measured individually and averaged for the sample for analysis.

To study the CI, mussels were sampled from each station on a monthly basis. During sampling, clumps of mussels were randomly collected from the three

sampling points in each station and transported to the laboratory in insulated box. The samples from each station were declumped for pooling together and condition index of the mussel sub-sample (n=30 to 35) were analysed according to Crosby and Gale (1990).

Mussels were cleaned, measured (using a digital Vernier calipers, ± 0.01 mm) and whole live weight and volume; shucked dry shell volume and weight; shucked wet meat weight and dry (80°C, 48 h) soft tissue weight were determined for each individual mussel and the condition index was estimated. The total volume (ml) and shell volume (ml) was found by displacement method and the meat was shucked by cold shucking method.

3.3.5. Data treatment

The monthly mean CI and the annual CI of the beds were calculated for each mussel bed. Monthly mean, maximum and minimum of CI and environmental variables of mussel beds were calculated for analysis.

To evaluate the variation of monthly CI values with reference to the mean CI during the study period, a CI ratio was calculated as:

$$\text{CI ratio} = \bar{x}\ CI_{month} / (\bar{x} CI_{all\ months}).$$

Based on the values obtained, the monthly CI for each station which exceeded its annual mean was classified as "high (CI ratio ≥1)" and the remaining as "low (CI ratio <1)" (Hickman *et al.*, 1991).

3.3.6. Statistical analysis

The seasonal and spatial variations in seston and condition index of the mussel beds were analysed using two-way ANOVA. When significant differences between means were detected, post factors were further analysed using a Range test, Student-Newman-Keuls (S-N-K) tests set at 5% significance level to identify homogeneous subsets of means using SPSS (13.0) software. For studying the seasonal variations, monthly data were classified into three seasons *viz.*, pre-monsoon (February to May), monsoon (June to September) and post-monsoon (October to January).

Principal Component Analysis (PCA) was used to determine the dominant patterns of change in environmental parameters (temperature, salinity, pH, DO, rainfall, chl-a, POM, SPM, PIM, POM/SPM, chl-a/POM, PIM%) of the mussel beds in order to explain the importance of each element in the overall variability. Principal Component Analysis (PCA) known as empirical orthogonal function analysis objectively extracts the optimal linear lower-dimensional structure from a multivariate dataset. The usefulness of PCA in condensing and interpreting multivariate water quality data have been demonstrated by Kaplunovsky (2005). The analysis is successful if few Principal Components (PC) explains large

proportion of the data set variation. The variance of environmental factors was reduced using PCA and only Principal Components with eigenvalues exceeding 1 were analysed with Varimax rotation. PCA was done for the parameters of each mussel beds separately.

Multiple stepwise regression analysis was conducted to assess the effects of environmental variable on CI. The regression analysis was carried out with the monthly CI ratio and the monthly environmental variables. CI measured at the end of a particular month represents the effects of average habitat conditions over the entire month (Brown and Hartwick, 1988) therefore, environmental variables were entered into the regression analysis as monthly values. Potential predictable variables were water temperature, salinity, chl-a, rainfall, POM, SPM, POM/SPM, chl-a/POM. The analysis was done with log values of the data in order to minimize the influence of extreme values.

Stepwise discriminant analysis was conducted to study whether mussels had distinctive condition status profiles with variations in quantity or quality of seston along with physico-chemical parameters. Discriminant analysis (Seal, 1964) is a classification method that measures the important factors determining membership within a category. In the present study, it was used to predict CI discrimination accuracy between CI_{high} and CI_{low} using SPSS (13.0) software. The success of the discriminant analysis was evaluated by the percentage of correctly classified cases.

3.4. Results

3.4.1. Seston availability in mussel beds

The seston availability and temporal variations of seston in the mussel beds off Someshwara and Surathkal are presented in Table 3.1 and Table 3.2 respectively. ANOVA revealed no significant variations between the surface and bottom levels of seston in seawater and therefore the depth-wise data was pooled for further analysis.

3.4.1.1. Chlorophyll-a

Someshwara: Chl-a concentrations ranged from 2.70 mg/m^3 in June to 28.42 mg/m^3 in September with a mean of 6.79±5.85 mg/m^3. March and September witnessed high concentrations of chl-a. The seasonal variations of chl-a levels are given in Fig. 3.1. Chl-a content showed a clear seasonal trend, with maximum during monsoon (10.22 mg/m^3) and minimum during post-monsoon (4.37 mg/m^3).

Surathkal: The mean chl-a levels off Surathkal was 6.43±2.51 mg/m^3. The month-wise analysis indicated high levels in March (13.39 mg/m^3) and September (11.78 mg/m^3) and low in December (4.34 mg/m^3) (Table 3.2). The seasonal variations of chl-a are presented in Fig. 3.1. Chl-a content off Surathkal (Table 3.3) was highest during monsoon (7.09 mg/m^3) followed by pre-monsoon (6.79 mg/m^3) and post-monsoon (5.26 mg/m^3). Seasonal and spatial variations in chl-a levels analysed by two-way ANOVA (Table 3.4) indicated statistically significant difference between seasons ($p<0.05$). However, chl-a levels showed no significant differences between stations. Post-hoc analysis using S-N-K test among seasons revealed significantly higher mean chl-a content during monsoon (Table 3.3).

3.4.1.2. Particulate Organic Matter (POM)

Someshwara: Wide variations were noticed in the level of POM in the mussel beds of the study area (Table 3.1). In Someshwara, it varied between 3.18 mg/l in January and 15.27 mg/l in September (6.29±5.05 mg/l). The POM content measured at 3.18 mg/l in January increased to 5.20 mg/l in March and declined by April-May. It registered an increasing trend with the progress of monsoon. In June the values reached 6.20 mg/l and by July it rose to 14.04 mg/l. The organic matter after registering a peak in September started declining in the post-monsoon months. Analysis of seasonal variation in POM indicated highest POM levels during monsoon followed by pre-monsoon and post-monsoon (Fig. 3.1).

***Surathkal*:** POM levels at Surathkal ranged from 2.72 mg/l in November to 21.13 mg/l in June with a mean of 6.45±4.92 mg/l. POM levels were highest during monsoon followed by pre-monsoon and post-monsoon (Fig. 3.1).

Analysis of seasonal and spatial changes using two-way ANOVA indicated significant ($p<0.05$) variation among seasons (Table 3.5). Post-hoc comparisons indicated that the POM was significantly ($p<0.05$) higher in monsoon among the three seasons (Fig.3.1, Table 3.3). No significant difference in POM levels was observed between the stations.

3.4.1.3. Suspended Particulate Matter (SPM)

***Someshwara*:** Suspended particulate matter includes organic and inorganic material that becomes suspended in water causing turbidity of water. Monthly variation of SPM in mussel beds of Someshwara is presented in Table 3.1. The mean SPM level was 30.32±17.03 mg/l and it varied between a minimum of 16.14 mg/l in January and maximum of 56.38 mg/l in July (Fig. 3.2). Seasonal analysis of SPM at Someshwara indicated highest values in monsoon followed by pre-monsoon and post-monsoon (Table 3.3).

Surathkal: The SPM level of Surathkal mussel beds showed wide variations (Table 3.2). It ranged between 11.80 mg/l in December and 114.73 mg/l in June with a mean of 30.31±25.43 (Fig. 3.2). Seasonal analysis indicated significant difference in SPM levels between seasons with the highest during monsoon followed by pre-monsoon and post-monsoon.

Two-way ANOVA indicated significant variations in SPM between seasons while no significant variation was observed between the two stations (Table 3.6). Post-hoc S-N-K tests indicated that the monsoon had significantly higher SPM levels relative to other seasons (Table 3.3).

3.4.1.4. Particulate Inorganic Matter (PIM)

Someshwara: Monthly variations in PIM of the mussel beds are presented in Table 3.1. Mean PIM in the mussel beds of Someshwara was 24.89±13.59. It ranged between 12.96 mg/l in January and 42.34 mg/l in July. Season-wise analysis of PIM of indicated highest mean value during monsoon (34.97 mg/l) and the lowest during post-monsoon (17.65 mg/l) (Fig. 3.2).

Surathkal: PIM levels in mussel beds of Surathkal showed wide variations with a mean of 23.85±20.81 (Table 3.2). The lowest value observed was 8.55 mg/l in December and the highest was 93.60 mg/l in June. Highest mean PIM was observed during monsoon and the lowest during post-monsoon.

PIM levels of the mussel beds were significantly higher in monsoon (S-N-K analysis) when compared to the pre-monsoon and post-monsoon season (Table

3.3). No significant difference was observed between stations with regard to the PIM levels (Table 3.7).

3.4.2. Quality of seston in mussel beds

Someshwara: The organic fraction of seston, POM/SPM varied from 0.13 (November) to 0.35 (September), while the ratio of chl-a/POM varied from 0.46 (July) to 2.73 (October) at Someshwara (Table 3.1).

Surathkal: The POM/SPM ratio in Surathkal mussel bed was found to be high subsequent to the monsoon and it varied from 0.17 in February to 0.30 in October (Fig. 3.3). The chl-a/POM ratio was found varying between 0.29 (June) and 3.33 (February) (Table 3.2).

The two-way ANOVA for the seasonal differences in POM/SPM ratio and chl-a/POM ratio are provided in Table 3.8 and Table 3.9 respectively. There were significant seasonal differences in the organic content of the seston (POM/SPM) between the stations as well as seasons. Significant differences were not observed in the chl-a/POM values of the mussel beds on a temporal or spatial scale.

The PIM fraction of the seston in Someshwara and Surathkal mussel beds is presented in Table 3.1 and Table 3.2 respectively. The percentage of PIM was less in September at Someshwara, while at Surathkal it was lowest in October.

3.4.3. Spatial variability of environmental parameters in mussel beds

Someshwara: Principal Component Analysis extracted four principal axes with eigenvalues higher than 1. The eigenvalues and cumulative percentage of variance are presented in Table 3.10. The first four principal components (PC) are responsible for more than 87.02% of the data scatter in mussel beds off Someshwara. Based on PCs eigenvalues, the number of evaluated PCs was limited to the first four. Distribution of PC1 and PC2 loadings are presented as a scatterplot of individual variables in space spanned by axis PC1 and PC2 separately for the mussel beds (Fig. 3.4). It can be seen from the table that variables like salinity, temperature, pH, DO and rainfall exhibit PC1 loadings exceeding 0.6 and explained 43.75% of the variance. PC1 score was positively correlated with the salinity, temperature, pH and negatively with the rainfall and DO. PC2 explained 21.1% of the variance, represented by the organic content of the seston together with the percentage of inorganic suspended particles. PC3 explained 12.2% of the variance with eigenvalue of 1.47, comprising POM, SPM and PIM. The fourth component (PC4) explained 10% of the variance (eigenvalue 1.19) with Chl-a exhibiting the highest loading on PC4 followed by the ratio of chl-a to the POM.

Table 3.1. Variations (mean ± SD) in seston quantity [chlorophyll-a (chl-a), particulate organic matter (POM), particulate inorganic matter (PIM) and suspended particulate matter (SPM)] and quality [percentage of PIM in SPM (% PIM), chlorophyll-a to particulate organic matter ratio (Chl-a/POM) and particulate organic matter to suspended particulate matter ratio (POM/SPM)] in mussel beds off Someshwara.

Month	Chl-a (mg/m^3)	POM (mg/l)	PIM (mg/l)	SPM (mg/l)	% PIM	Chl-a/POM	POM/SPM
Jan	4.09±1.44	3.18±1.62	12.96±7.03	16.14±8.65	79.84±1.48	1.97±1.56	0.20±0.01
Feb	4.23±1.63	4.26±2.30	18.95±17.89	23.22±20.08	77.22±8.19	1.11±0.41	0.23±0.08
Mar	8.43±1.53	5.2±1.54	27.08±10.35	32.29±11.73	83.34±2.57	1.82±0.52	0.17±0.03
Apr	4.91±0.82	3.63±3.31	16.47±4.05	16.26±7.37	84.19±10.36	2.59±2.25	0.16±0.10
May	5.90±0.56	3.93±0.39	17.77±1.48	21.70±1.73	81.86±1.24	1.52±0.19	0.18±0.01
Jun	2.70±0.89	6.2±1.58	31.8±8.58	38.00±10.14	83.59±0.75	0.48±0.26	0.16±0.01
Jul	6.26±2.90	14.04±7.08	42.34±7.91	56.38±5.16	75.06±11.80	0.46±0.13	0.25±0.12
Aug	7.46±0.41	5.51±1.56	29.47±12.31	34.96±13.77	83.40±3.36	1.46±0.46	0.17±0.03
Sep	28.42±3.01	15.27±2.94	28.93±5.06	44.20±6.36	65.29±5.55	1.90±0.29	0.35±0.06
Oct	8.46±4.24	3.80±1.24	24.13±8.91	27.93±9.80	85.84±3.84	2.72±2.36	0.14±0.04
Nov	2.92±2.41	4.15±2.42	26.6±11.68	30.75±14.02	87.03±2.08	1.00±0.77	0.13±0.02
Dec	3.70±1.47	4.36±2.96	19.84±16.60	24.20±19.04	80.02±7.76	1.08±0.54	0.20±0.08
Mean	6.79±5.85	6.29±5.05	24.89±13.59	30.32±17.03	79.99±8.11	1.45±1.17	0.20±0.08

Table 3.2. Variations (mean ± SD) in seston quantity [chlorophyll-a (chl-a), particulate organic matter (POM), particulate inorganic matter (PIM) and suspended particulate matter (SPM)] and quality [percentage of PIM in SPM (% PIM), chlorophyll-a to particulate organic matter ratio (Chl-a/POM) and particulate organic matter to suspended particulate matter ratio (POM/SPM)] in mussel beds off Surathkal.

Month	Chl-a (mg/m^3)	POM (mg/l)	PIM (mg/l)	SPM (mg/l)	% PIM	Chl-a/POM	POM/SPM
Jan	6.09±0.85	4.05±1.24	10.13±1.57	14.18±1.16	71.35±8.81	1.60±0.41	0.29±0.09
Feb	4.98±1.06	7.00±6.33	24.32±17.43	31.31±23.56	82.50±8.22	3.33±4.26	0.17±0.08
Mar	13.39±1.31	6.1±0.5	21.03±2.31	27.13±2.57	77.44±1.81	2.21±0.30	0.23±0.02
Apr	7.08±0.91	7.36±2.42	32.00±10.14	39.37±12.50	81.29±1.35	1.02±0.23	0.19±0.01
May	5.45±0.34	4.58±0.54	14.34±3.00	18.92±2.90	75.17±5.00	1.20±0.14	0.25±0.05
Jun	6.10±0.74	21.13±2.86	93.60±9.71	114.73±9.52	81.49±2.75	0.29±0.06	0.19±0.03
Jul	5.77±1.48	8.90±3.75	33.82±12.83	42.72±16.51	79.40±1.65	0.88±0.63	0.21±0.02
Aug	6.57±0.86	3.96±1.72	17.97±4.76	21.93±5.11	81.78±8.21	3.00±4.68	0.18±0.08
Sep	11.78±0.47	6.87±1.06	23.13±2.94	30.00±3.85	77.13±1.79	1.754±0.32	0.23±0.02
Oct	4.80±0.30	6.64±1.32	15.47±3.82	22.10±5.07	69.71±2.34	0.75±0.15	0.30±0.02
Nov	4.93±1.48	2.72±1.34	12.88±9.36	15.60±10.64	80.85±5.24	2.08±0.81	0.19±0.05
Dec	4.34±0.69	3.25±1.26	8.55±1.62	11.80±1.25	72.42±11.00	1.49±0.55	0.28±0.11
Mean	6.43±2.51	6.45±4.92	23.85±20.81	30.31±25.43	77.91±7.10	1.76±2.29	0.22±0.07

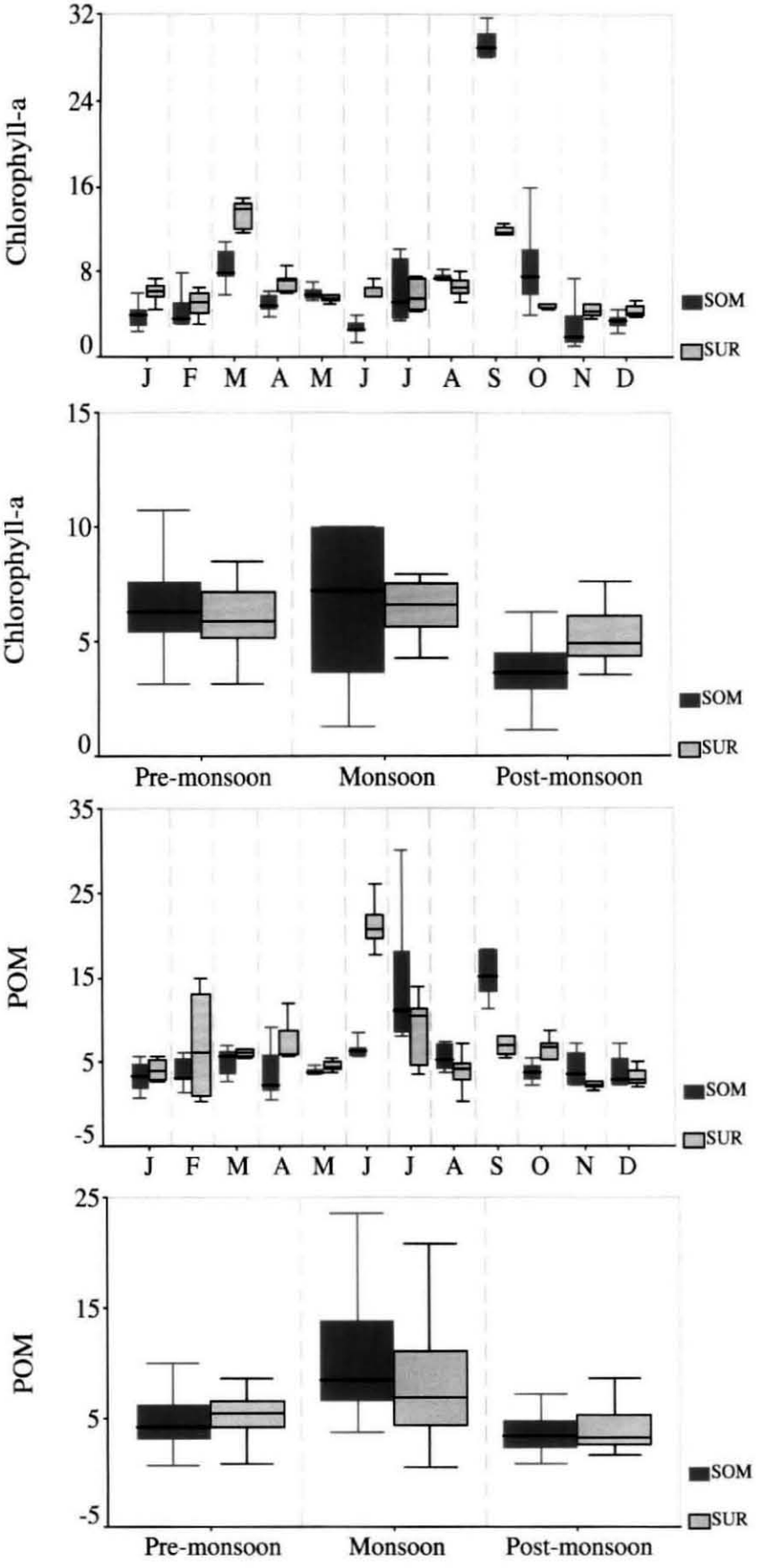

Fig. 3.1. Box and Whisker summary plot based on the median, quartiles and extreme values representing the seasonal trends in chlorophyll-a (mg/m^3) and particulate organic matter (POM mg/l) levels in mussel beds off Someshwara (SOM) and Surathkal (SUR).

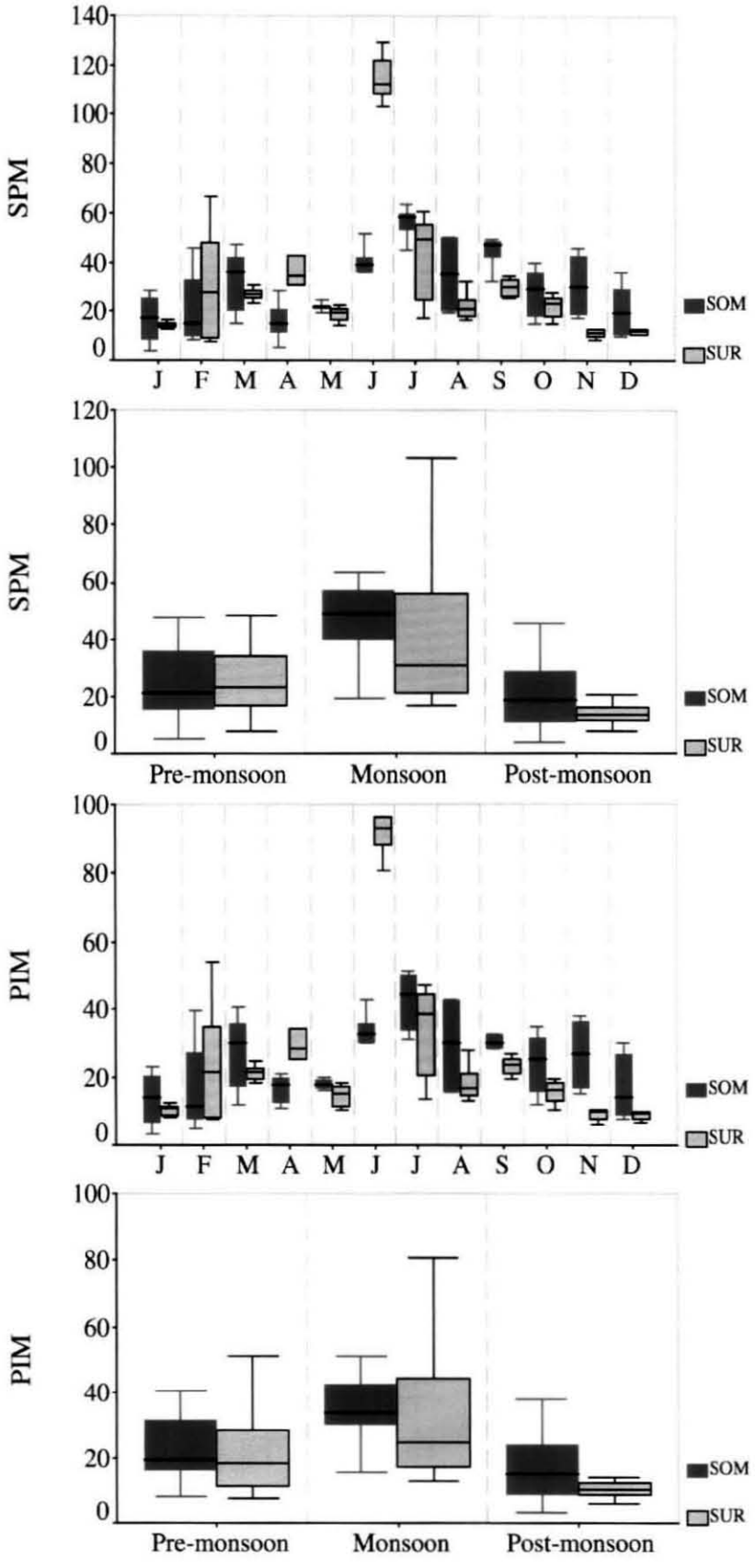

Fig. 3.2. Box and Whisker summary plot based on the median, quartiles and extreme values representing the seasonal trends in suspended particulate matter (SPM mg/l) and particulate inorganic matter (PIM mg/l) levels in mussel beds off Someshwara (SOM) and Surathkal (SUR).

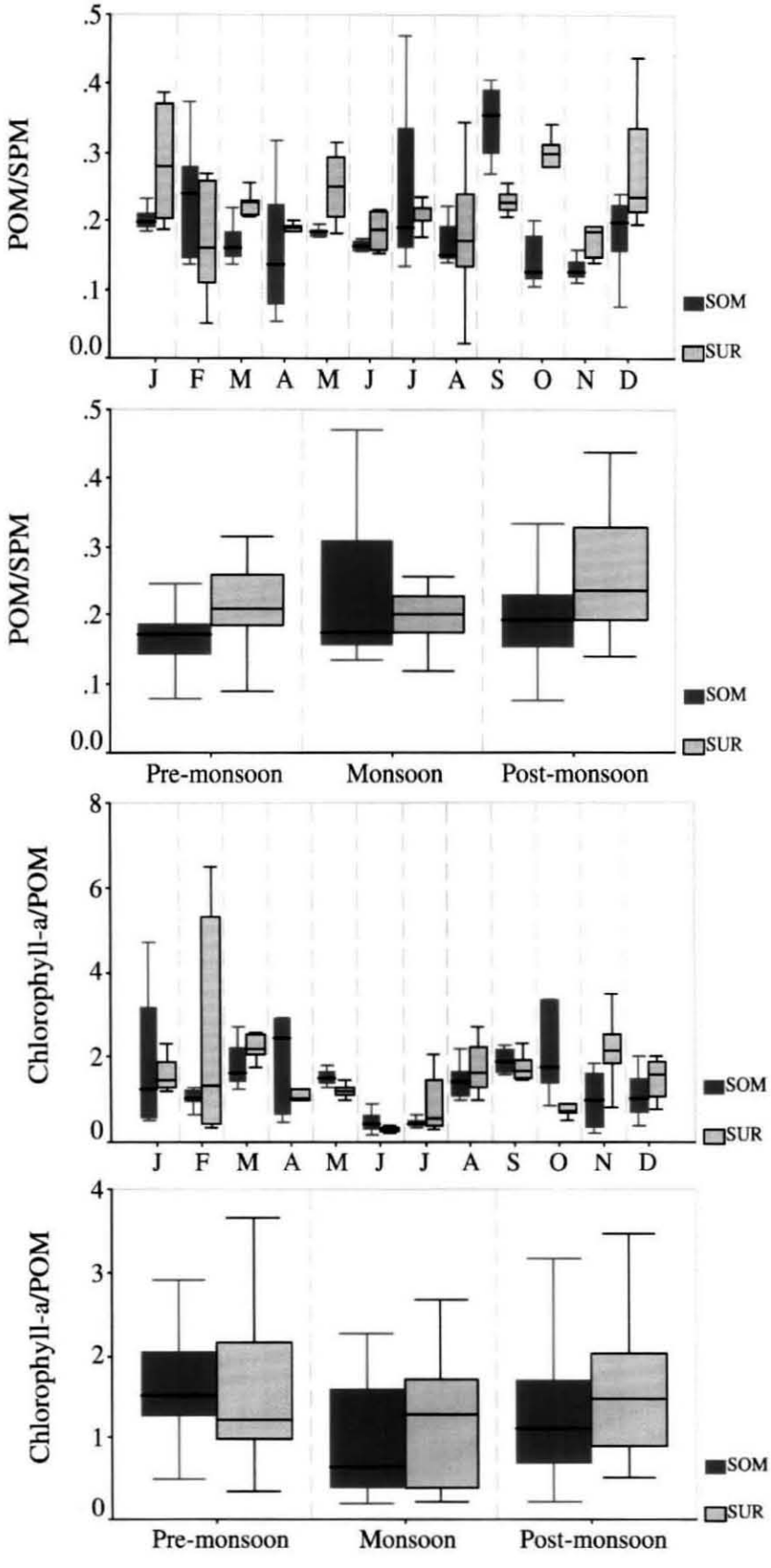

Fig. 3.3. Box and Whisker summary plot based on the median, quartiles and extreme values representing the seasonal trends in the ratio of particulate organic matter/suspended particulate matter (POM/SPM) and chlorophyll-a/particulate organic matter (chlorophyll-a/POM) levels in mussel beds off Someshwara (SOM) and Surathkal (SUR).

Table 3.3. Seasonal variations (mean ± SD) of chlorophyll-a (chl-a), particulate organic matter (POM), particulate inorganic matter (PIM) and suspended particulate matter (SPM) in mussel beds off Someshwara and Surathkal with results of S-N-K post-hoc tests.

Season	Chl-a (mg/m^3)	POM (mg/l)	SPM (mg/l)	PIM (mg/l)
Someshwara				
Pre-monsoon	6.52±1.99 [a]	4.58±2.23 [a]	25.91±14.50 [a]	23.39±12.32 [a]
Monsoon	10.22±9.65 [b]	11.01±6.32 [b]	45.98±12.29 [b]	34.98±10.26 [b]
Post-monsoon	4.37±2.76 [c]	3.79±1.99 [c]	21.43±13.64 [c]	17.65±12.03 [c]
Surathkal				
Pre-monsoon	6.79±3.11 [a]	6.11±3.85 [a]	27.83±15.96 [a]	21.72±12.37 [a]
Monsoon	7.09±2.37 [b]	8.96±6.43 [b]	45.67±34.13 [b]	36.71±27.98 [b]
Post-monsoon	5.26±1.18 [c]	4.02±1.85 [c]	15.81±6.95 [c]	11.79±5.86 [c]

Note: Seasonal means with the different letter denotes groups which are significantly different ($p<0.05$).

Table 3.4. Analysis of variance of chl-a concentrations between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	507	3	169	**9.5**	*0.000*
	Station	23	1	23	1.3	*0.258*
	Season	497	2	248	**13.9**	*0.000*
2-Way Interactions	Station x Season	158	2	79	**4.4**	*0.013*
Model		649	5	130	**7.3**	*0.000*
Residual		3747	210	18		
Total		4396	215	20		

Table 3.5. Analysis of variance of particulate organic matter levels between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	1337	3	446	**25**	*0.000*
	Station	0	1	0	0	*0.869*
	Season	1336	2	668	**37**	*0.000*
2-Way Interactions	Station x Season	108	2	54	3	*0.054*
Model		1403	5	281	**15**	*0.000*
Residual		3519	194	18		
Total		4922	199	25		

Table 3.6. Analysis of variance of suspended particulate matter levels between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	25843	3	8614	**24.7**	*0.000*
	Station	91	1	91	0.3	*0.610*
	Season	25843	2	12921	**37.1**	*0.000*
2-Way Interactions	Station x Season	514	2	257	0.7	*0.480*
Model		26307	5	5261	**15.1**	*0.000*
Residual		69022	198	349		
Total		95329	203	470		

Table 3.7. Analysis of variance of particulate inorganic matter levels between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	15188	3	5063	**21.2**	*0.000*
	Station	184	1	184	0.8	*0.381*
	Season	15143	2	7571	**31.7**	*0.000*
2-Way Interactions	Station x Season	483	2	241	1.0	*0.366*
Model		15831	5	3166	**13.2**	*0.000*
Residual		46363	194	239		
Total		62194	199	313		

Table 3.8. Analysis of variance of particulate organic matter (POM) to suspended particulate matter (SPM) ratio (POM/SPM) between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	p
Main Effects	(Combined)	0.076	3	0.025	**4.91**	*0.003*
	Station	0.024	1	0.024	**4.65**	*0.032*
	Season	0.056	2	0.028	**5.39**	*0.005*
2-Way Interactions	Station x Season	0.088	2	0.044	**8.54**	*0.000*
Model		0.159	5	0.032	**6.14**	*0.000*
Residual		1.005	194	0.005		
Total		1.164	199	0.006		

Table 3.9. Analysis of variance of chlorophyll-a (chl-a) to particulate organic matter (POM) ratio (Chl-a/POM) between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	18	3	6	1.8	*0.152*
	Station	5	1	5	1.4	*0.234*
	Season	13	2	7	1.9	*0.146*
2-Way Interactions	Station x Season	4	2	2	0.6	*0.559*
Model		21	5	4	1.3	*0.286*
Residual		656	194	3		
Total		677	199	3		

Table 3.10. Results of Principal Component Analysis of environmental parameters [rainfall, seawater temperature, salinity, pH, dissolved oxygen (DO), chlorophyll-a (chl-a), particulate organic matter (POM), particulate inorganic matter (PIM), suspended particulate matter (SPM), percentage of PIM in SPM (% PIM), chlorophyll-a to particulate organic matter ratio (Chl-a/POM) and particulate organic matter to suspended particulate matter ratio (POM/SPM)] in the mussel beds off Someshwara and Surathkal.

Variable loadings: **Someshwara**		PC 1	PC 2	PC 3	PC4
Salinity	Physico-chemical	**0.868**		-0.296	0.24
Rainfall	parameters	**-0.864**		0.271	-0.218
pH		**0.837**	-0.103	-0.303	-0.153
DO		**-0.754**	0.215		
Temperature		**0.702**	-0.235	-0.182	-0.142
POM/SPM	Quality of Seston	-0.121	**0.984**		
PIM%		0.121	**-0.984**		
POM	Quantity of Seston	-0.288	**0.65**	**0.637**	
SPM		-0.297		**0.945**	
PIM		-0.262	-0.174	**0.937**	
Chl-a	Primary production		0.45	0.311	**0.782**
Chl-a/POM		0.166	-0.288	-0.426	**0.754**
Eigenvalues		5.247	2.531	1.474	1.191
% of Variance		43.725	21.094	12.28	9.921
Cumulative %		43.725	64.819	77.099	87.02
Rotation Method: Varimax with Kaiser Normalization.					

Variable loadings: **Surathkal**		PC 1	PC 2	PC 3	PC4
SPM	Quantity of Seston	**0.982**	-0.13		
PIM		**0.976**	-0.132	0.117	
POM		**0.947**	-0.116	-0.192	
Salinity	Physico-chemical	-0.151	**0.923**	-0.149	
Temperature	parameters	-0.1	**0.896**		
pH		-0.268	**0.772**	-0.21	0.168
Rainfall		0.626	**-0.668**	0.157	
DO		-0.113	**-0.491**	-0.246	0.161
POM/SPM	Quality of Seston			**-0.974**	
PIM%				**0.974**	
Chl-a/POM	Primary production	-0.439		**0.636**	**0.239**
Chl-a					**0.963**
Eigenvalues		4.478	2.555	2.012	1.046
% of Variance		37.318	21.289	16.764	8.715
Cumulative %		37.318	58.607	75.371	84.086
Rotation Method: Varimax with Kaiser Normalization.					

Note: Factors with the highest loading on each principal component (PC) are shown in bold.

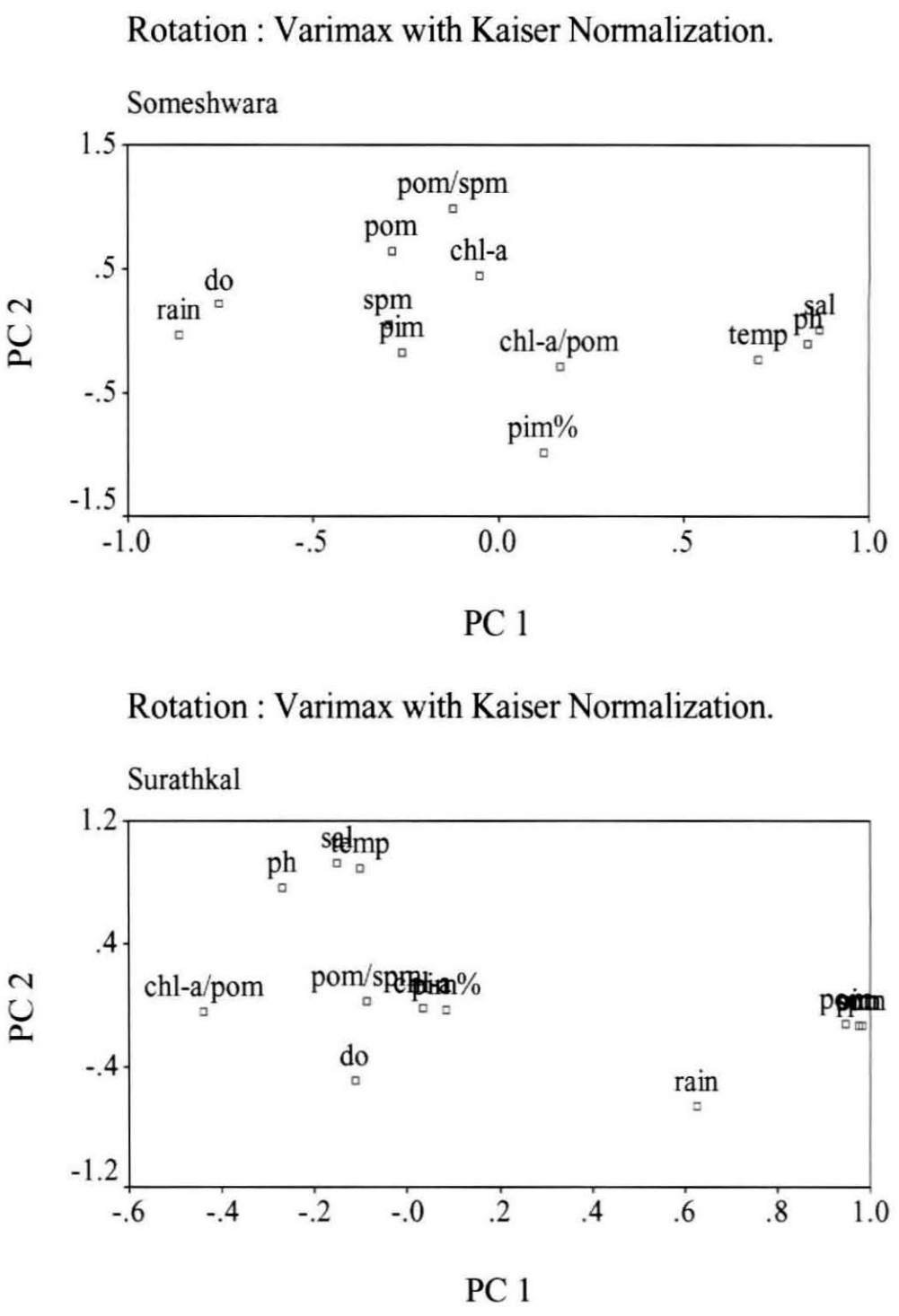

Fig. 3.4. Scatter plots showing the results of the Principal Component Analysis (PC 1 and PC 2) of environmental variables [rainfall (rain), temperature (temp), salinity (sal), dissolved oxygen (DO), pH, chlorophyll-a (chl-a), particulate organic matter (POM), suspended particulate matter (SPM), particulate inorganic matter (PIM), percentage of PIM (% PIM), particulate organic matter/suspended particulate matter (POM/SPM), chlorophyll-a/particulate organic matter (chl-a/POM)] off Someshwara and Surathkal mussel beds.

Surathkal: The environmental variables were reduced to four principal components by PCA with eigenvalue higher than 1 explaining 84.08% of the variability. The quantity of seston along with variables SPM, POM and PIM was identified as the most important component (PC1) accounting for 37.31% of variance, followed by the physico-chemical factors (PC2) such as salinity, water temperature, pH, rainfall and DO, together accounting for 21.28% variance (Table 3.10). PC1 score was positively correlated with PIM, POM and SPM which also showed positive correlation with precipitation. PC3 and PC4 were built from variables indicative of the organic fraction of the seston as well as the primary production accounting for 16.76 and 8.71% of variability respectively. In both the stations, about 60% of the data variance could be explained by the first two principle components.

3.4.4. Condition index and percentage edibility

CI_{grav} of mussels ranged from 56.43 in July to 124.23 in March (Fig. 3.5) at Someshwara and at Surathkal it ranged from 41 in June to 186 in March (Table 3.11). The average CI of the mussels from the Someshwara was 94.06±36.31 and Surathkal 123.21±49.82. Table 3.12 presents the seasonwise mean CI of mussels at Someshwara and Surathkal. Results of the two-way ANOVA are presented in Table 3.13. The CI was significantly different between stations and seasons ($p<0.05$). Although differences in CI were significant among the mussel beds, it displayed similar patterns in annual cycles with distinct peaks in March and September. CI displayed continuous decline from May to July which later improved from August. CI was highest in Pre-monsoon at Someshwara (105.75) whereas at Surathkal better condition was observed during the pre-monsoon (134.92) as well as post-monsoon seasons (142.22) (Fig.3.5). Post-hoc analysis showed significant difference between the condition indices of mussels in the three seasons (Table 3.12).

Meat yield or percentage edibility also displayed similar patterns as CI among the two mussel beds. Distinct peaks in percentage edibility were observed in September and March (Table 3.11). Variation in the percentage edibility of mussels is shown in Fig. 3.5. Generally, percentage edibility of mussels was low in June to July and high in March and September-October months. At Someshwara the highest (27.57%) and the lowest (18.06%) values were recorded at the end of September and January respectively. At Surathkal, the lowest meat percentage was in July (15.33%) and the highest in September (32.65%). Two-way analysis of variance indicated significant variations ($p<0.05$) in average meat percentage between seasons and stations (Table 3.14).

3.4.5. Influence of environmental parameters on condition index

Stepwise multiple regression analysis was used to study the relationship between CI and environmental variables. The regression analysis done for each of the stations independently and for both the stations combined yielded identical results. Multiple regression analysis showed that the most significant variables contributing to increase in the CI were the increase in water temperature, POM and chl-a content.

In the mussel beds off Dakshina Kannada, water temperature was the most significant variable in the regression equation where it explained 35% of the variation in CI. Seston content (POM and chl-a) of the mussel beds along with water temperature explained 56% of the variations in CI (Table 3.15).

Discriminant analysis was performed to discriminate distinctive condition status profiles in mussels having high CI ratio (CI_{high}) and low CI ratio (CI_{low}) upon changes in environmental parameters. The parameters weighed high in the model for the referred differentiation for CI were chl-a and temperature. The CI_{high} exhibited the greatest discriminatory success. Analysis of the monthly CI ratio, mean water temperature and Chl-a levels (Fig. 3.6) showed that 83.9% of the CI_{high} was associated with high chl-a and high water temperature group whereas, 72.4% of CI_{low} were associated with low chl-a and low water temperature regime (Table 3.16).

Table 3.11. Variations (mean ± SD) of condition index (CI) and percentage edibility (% Edibility) of mussels from mussel beds off Someshwara and Surathkal.

Month	Someshwara		Surathkal	
	CI_{grav}	% Edibility	CI_{grav}	% Edibility
Jan	68.32±30.57	18.06±3.84	137.14±27.15	25.15±3.45
Feb	76.04±22.83	20.10±3.38	103.51±41.82	25.14±7.14
Mar	124.22±36.50	26.80±4.90	186.59±31.79	29.63±3.11
Apr	96.23±37.07	21.38±5.56	140.73±38.12	28.55±4.68
May	117.60±33.68	24.69±5.32	118.39±26.33	26.20±3.39
Jun	70.18±20.94	24.65±3.58	41.00±09.62	16.14±2.31
Jul	56.42±22.42	20.02±6.60	44.09±08.49	15.33±1.78
Aug	104.68±29.03	23.48±3.30	79.45±24.35	20.40±4.16
Sep	112.36±24.24	27.57±4.92	145.00±47.58	32.65±7.83
Oct	92.53±29.63	22.13±3.12	130.42±44.34	27.20±5.99
Nov	110.44±25.12	24.03±3.25	121.82±25.70	26.24±4.23
Dec	91.10±28.04	20.18±4.73	153.28±28.54	30.08±3.97
Mean	94.06±36.31	21.91±5.34	123.21±49.83	26.50±7.05

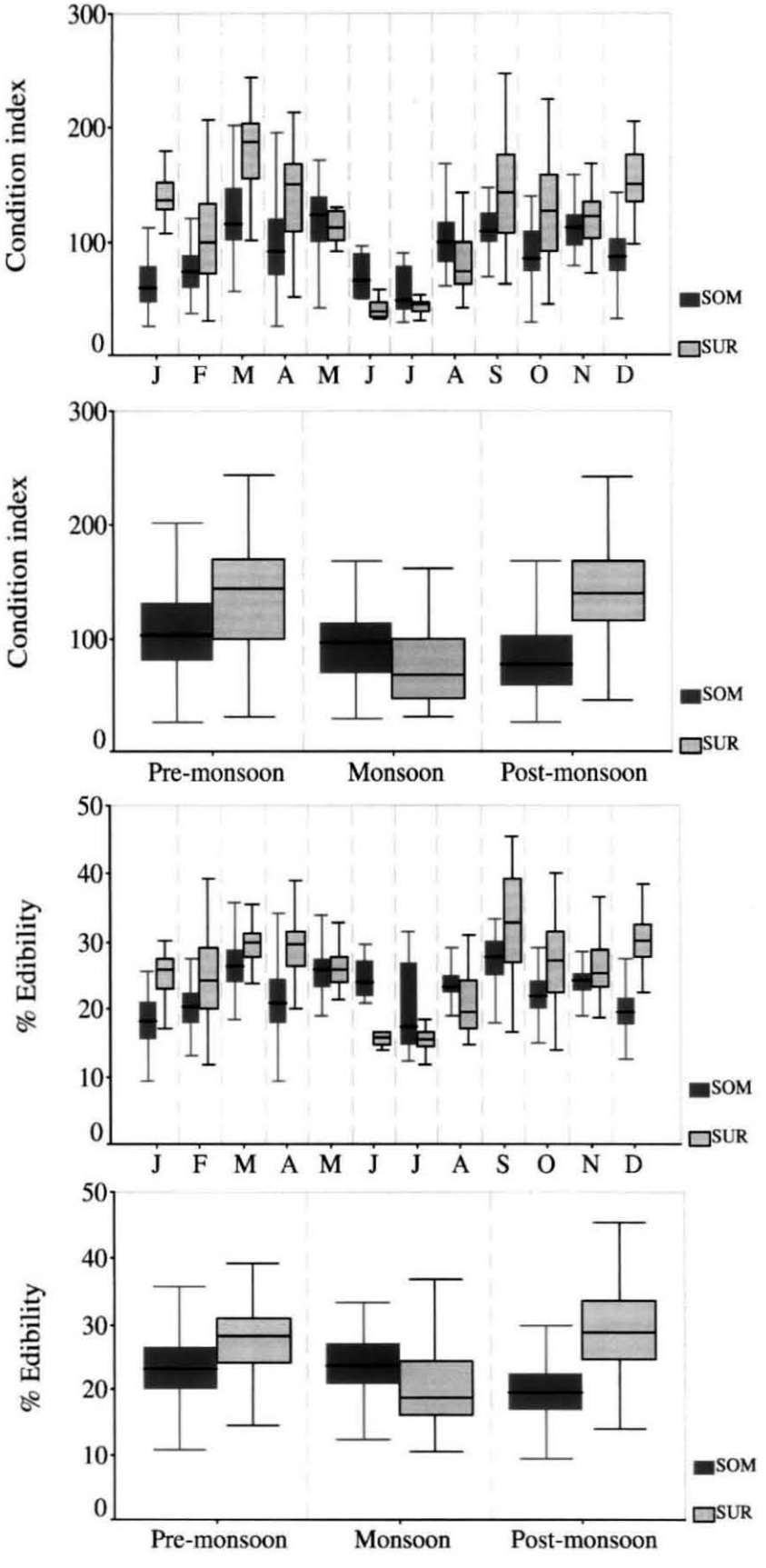

Fig. 3.5. Box and Whisker summary plot based on the median, quartiles and extreme values representing the seasonal trends in condition index and percentage edibility of mussels from Someshwara (SOM) and Surathkal (SUR) mussel beds.

Table 3.12. Seasonal variations (mean ± SD) of condition index (CI) and percentage edibility (% Edibility) of mussels from mussel beds off Someshwara and Surathkal with results of S-N-K post-hoc tests.

Station	Season	Condition index	% Edibility
Someshwara	Pre-monsoon	105.75±37.31 [a]	23.37±5.56 [a]
	Monsoon	92.51±33.92 [b]	23.67±5.16 [b]
	Post-monsoon	82.17±31.89 [c]	19.82±4.39 [c]
Surathkal	Pre-monsoon	134.93±48.09 [a]	27.37±5.64 [a]
	Monsoon	77.15±37.85 [b]	20.36±5.69 [b]
	Post-monsoon	142.22±38.37 [c]	29.61±6.56 [c]

Note: Seasonal means with the different letter denotes groups that are significantly different ($p<0.05$).

Table 3.13. Analysis of variance of condition index of mussels between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	227384	3	75795	**52.4**	*0.000*
	Station	120429	1	120429	**83.2**	*0.000*
	Season	147743	2	73872	**51.0**	*0.000*
2-Way Interactions	Station x Season	168345	2	84173	**58.1**	*0.000*
Model		561263	5	112253	**77.5**	*0.000*
Residual		1362335	941	1448		
Total		1923598	946	2033		

Table 3.14. Analysis of variance of percentage edibility of mussels between pre-monsoon, monsoon and post-monsoon seasons and between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Main Effects	(Combined)	3235	3	1078	**35.7**	*0.000*
	Station	2449	1	2449	**81.1**	*0.000*
	Season	1343	2	672	**22.2**	*0.000*
2-Way Interactions	Station x Season	5182	2	2591	**85.8**	*0.000*
Model		12329	5	2466	**81.6**	*0.000*
Residual		28842	955	30		
Total		41171	960	43		

Table 3.15. Summary of multiple regression analysis relating condition index of *P. viridis* (pooled for Someshwara and Surathkal mussel beds) with water temperature (°C), particulate organic matter (POM) (mg/l) and chl-a (mg/m^3)

Variable	F	Adjusted cumulative R^2	*p*	Coefficients ± S.E.
Temperature	28.52	0.354	*<0.0001*	6.410 ± 1.142
POM	27.63	0.520	*<0.0001*	0.214 ± 0.087
Chl-a	21.44	0.563	*<0.0001*	0.301 ± 0.136

Table 3.16. Percentage of condition index (CI) classified according to discriminant analysis performed on the CI ratios of *P. viridis* (pooled for Someshwara and Surathkal mussel beds).

Predicted group membership for temperature and chl-a		
	CI_{low}	CI_{high}
CI_{low}	72.4	27.6
CI_{high}	16.1	83.9
Overall	78.3%	

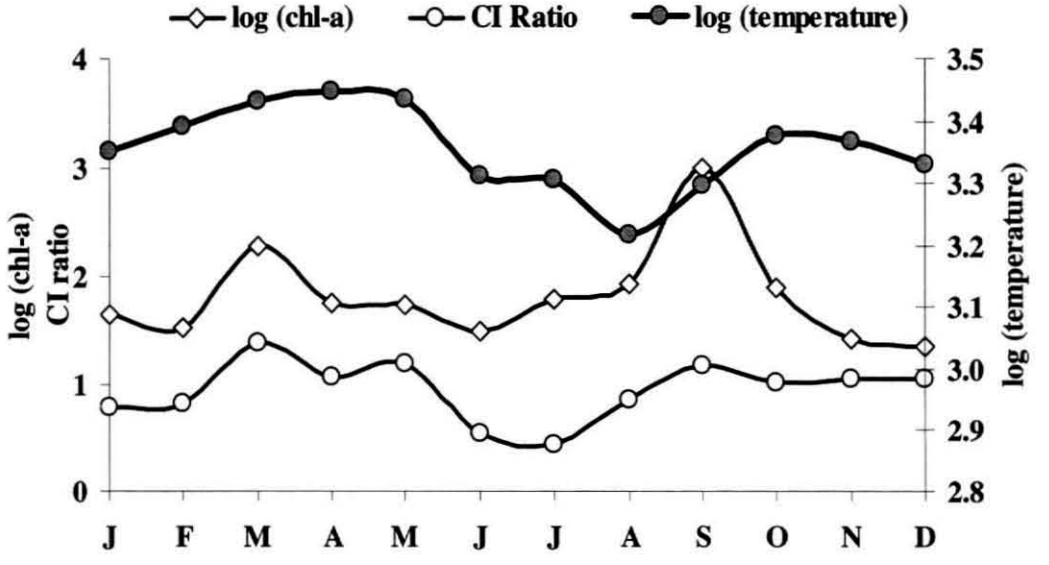

Fig. 3.6. Monthly condition index ratio ["high (CI ratio ≥1)" and "low (CI ratio <1)"] of *P. viridis* (CI ratio), chlorophyll-a (log chl-a) and water temperature (°C) of Someshwara and Surathkal mussel beds (pooled).

3.5. Discussion

3.5.1. Seston availability in mussel beds

Quantity and quality of the seston of mussel beds primarily decides the food available to the mussels and thereby largely influence the condition index of the mussels. Variation in the quantity and quality of seston in coastal waters has been attributed to numerous factors such as primary production, precipitation, proximity to river inflow and wind-induced resuspension of bottom material.

The phytoplankton biomass in the mussel beds off Dakshina Kannada presented a bimodal pattern with peak productions in March and September. The mean chl-a content of the mussel beds gradually increased from August and reached its maximum in September. Chl-a content was highest in monsoon followed by pre-monsoon and post-monsoon. Temporal variations in chl-a observed in the mussel beds were comparable with earlier observations from the region. Lingadhal *et al.*, (2003) while studying the primary productivity in Arabian Sea off Mangalore recorded bimodal pattern of distribution with peaks during the months of October/December and February/March and reported chl-a ranging from 0.84 to 23.22 mg/m^3. Krishnakumar and Bhat (2007) analysed the seasonal and interannual variations in oceanographic conditions off Mangalore coast for the period 1994 to 2004 and noticed peak primary production (chl-a) in the inshore waters in September (18.59 - 20.8 mg/m^3). Manjappa (1987), while describing the distribution of different chlorophyll pigments off Mangalore reported chl-a values ranging from 0.14 to 24.58 mg/m^3 with peak concentrations during January-February and in some stations during March. Similarly, Rivonker and Verlecar (1990), while working on the physico-chemical parameters of fishing grounds off Mangalore and Joseph *et al.* (1998), while conducting hydrochemical studies along the coastal waters off Surathkal, observed similar trend in seasonal variation of chl-a levels.

Localised phytoplankton production is dependent on the existing background nutrient concentrations and the degree of nutrient enrichment (Cheshuk *et al.*, 2003), as well as a range of other contributing factors including light intensity, photoperiod, temperature and salinity. In Arabian Sea, the magnitude of primary production is considerably influenced by upwelling. Upwelling during the south-west monsoon (Muraleedharan and Kumar, 1996) results in the nutrient enrichment of coastal waters influencing the primary production (Nair, 1974). Therefore, the phytoplankton biomass and productivity are higher in coastal regions during the south-west monsoon or immediately after it (Banse, 1996).

However, in June and July even with the increased availability of nutrients, the primary production in mussel beds was comparatively low when compared to August and September, probably due to increased cloudiness during these months reducing the light intensity.

During the later part of monsoon, in September, increased nutrient levels brought by upwelling and land runoffs and a steady light intensity increased the primary production resulting in a high chl-a content. Higher levels of chl-a in the coastal and near-shore waters of the west coast after monsoon have been reported by Devassy (1983) and Balachandran *et al.* (1989). Similarly, Bhattathiri *et al.* (1996), de Sousa *et al.* (1996) and Krishnakumar and Bhat (2007) observed high nutrient concentration and primary production in inshore waters of Mangalore during the same period.

Following the peak in the late monsoon, chl-a values in the seawater gradually decreased in post-monsoon. This may be due to a decrease in land runoffs and increased vertical stability of the coastal waters during the post-monsoon.

Besides phytoplankton, the organic and inorganic suspended particulate matter in the seawater also registered seasonal variations with high levels of SPM, POM and PIM during monsoon. Though studies on the levels of POM in mussel beds of Dakshina Kannada coast are limited, Padiyar (1999) working on the levels of suspended particles in Mulki estuary reported high seasonal variability in SPM ranging from 13 to 143 mg/l. These levels are comparable with the seasonal trend observed in the present study. Increased particle flux in the coastal waters has been linked with variations in river runoff, turbulence and changes in primary production (Rao, 1985).

Tidal currents and wind induced waves are also reported as prominent factors associated with resuspension and transport of sediments and associated organic particles into estuaries and riverine inflow has a potential for transporting significant quantity of these particulate material to the coastal waters (Demers *et al.*, 1987, de Jonge and van Beusekom, 1995). Along the west coast, variations in hydrography and nutrient profile have been reported to influence the quantity and composition of TPM, organic fraction of TPM and chl-a fluxes (Bhaskar *et al.*, 2000).

Seston distribution within the monsoon season was characterised by relatively high values for SPM, POM and PIM in June-July with the onset of monsoon. However, increase in chl-a levels was noticed only by late monsoon. The increase in POM levels during June-July without a corresponding increase in chl-a content signifies the role of detrital component of POM during the period. Whereas, the increase in POM levels in September corresponded to the peak chl-a levels in the mussel bed, indicating that the level of POM during the period was clearly influenced by phytoplankton production.

Few studies have reported similar seasonal changes of particulate organic matter along the west coast of India. Krishnakumar and Bhat (2007) attributed the low light attenuation coefficient during September-October to high turbidity associated with increase in phytoplankton production. Verlecar and Qasim (1985) while estimating the particulate materials along with the chlorophyll in the coastal waters of Goa and the adjoining estuarine system observed a

maximum particulate organic carbon (POC) content during monsoon. Based on the changes in the living and non-living fractions in total POC, they inferred that during monsoon besides the living plankton, concentrations of POC was affected considerably by non-living detrital component. Similarly, Padiyar (1999) attributed higher SPM values in monsoon to terrigenous input.

An increase in PIM fraction of suspended particulate matter (SPM) due to resuspension of inorganic sediments also resulted in peak levels of SPM recorded in June-July. High turbidity in the coastal areas of Dakshina Kannada observed earlier by many workers has been attributed to increased input of suspended material into the coastal area with increase in precipitation, turbulence in inshore areas and increased primary production (Reddy and Hariharan, 1986 and Lingadhal, 1995).

Analysis of the seston contents revealed that there was no statistically significant difference between the two mussel beds with respect to the quantity of the seston (chl-a, SPM, POM). However, a comparison of the standard deviation of chl-a and POM among mussel beds showed greater dispersion in chl-a and POM levels at Someshwara which can be attributed to the proximity of Someshwara mussel bed to the river mouth.

3.5.2. Quality of seston in mussel beds

High chl-a/POM ratio is indicative of superior quality of organic seston due to its low detritus fraction (Garen *et al.*, 2004). Though significant difference in the quality of seston was not observed between the two stations, the ratio was comparatively higher at Surathkal than Someshwara. A better ratio seen at Surathkal was due to the relatively low volume of river runoffs causing reduced POM concentration.

Seasonal analysis indicated a decline in seston quality at Someshwara with the onset of monsoon, which improved later towards the end of monsoon, whereas, the seston quality was more or less identical in monsoon and post-monsoon seasons at Surathkal. Decrease in chl-a/POM ratio observed in monsoon season in both the locations suggests that detritus, rather than living phytoplankton was contributing largely to the organic content. Decrease in the ratio seems to be related to high turbidity which reduced light penetration is considerably resulting in low fraction of chl-a in the organic suspended matter pool during June-July (Qasim *et al.*, 1969). Besides the decline in primary production, substantial increase in POM levels brought in by increased river inflow, turbulent mixing and resuspension of organic suspended matter can result in a decrease in the ratio.

The ratio of organic fraction of the seston to the total suspended particulate material is an index of the proportion of desirable food available to the mussels in their habitat. The ratio of POM to SPM varied significantly between the mussel beds of Someshwara and Surathkal. Relatively higher POM to SPM ratio

was observed at Someshwara in monsoon, whereas at Surathkal higher POM fraction was observed in post-monsoon. Higher POM/SPM ratio is indicative of environment with relatively better food availability. The increase in POM/SPM ratio shows increased detrital or chl-a fraction in the environment or a decrease in SPM. At Surathkal, the increase in the ratio had a strong phytoplankton signature associated with primary production.

In summary, substantial variability in the quality and quantity of seston is noticed in the mussel beds. To a great extent most of these variations were brought in by the monsoon which changed the hydrography of the study area. In terms of food availability, the chl-a levels observed was in general consistent with the values reported in many inshore and coastal areas (Verlecar and Qasim, 1985, Verlecar, 2006; Rajagopal *et al.*, 1998b and Krishnakumar and Bhat, 2007). The levels of chl-a observed in the mussel beds were above the benchmark for phytoplankton abundance (2µg/l) used for grading "good growing conditions" in mussel growing waters (Inglis, 2000).

3.5.3. Spatial variability in environmental parameters

The principal component analysis enabled identification of spatial variation of environmental parameters of the two mussel beds along with the quantitative apportionment of each source to the total variability. The results indicated that the two beds were not affected by the variables in similar fashion. Principal component analysis classified the twelve environmental factors into four clusters or groups. In mussel beds off Someshwara, the most significant group forming the first principal component included the physico-chemical parameters of the mussel bed, whereas at Surathkal, availability of seston, SPM, PIM and POM were the most important.

Mussel beds of Someshwara experienced highest variability of environmental parameters and it can probably be attributed to the proximity of the site (5.5 km south of Nethravati-Gurpur barmouth) to the mouth of River Nethravati. The Nethravati River discharges huge volumes of fresh water, which largely influence the hydrobiology of the estuary and inshore areas. The river flow is stronger during the monsoon and post-monsoon corresponding to the increase in precipitation over the catchment area. Though riverine inflow has a potential for transporting significant quantity of particulate material to the coastal waters, at Someshwara, the variations in seawater temperature and salinity associated with precipitation and river-runoff were the most significant factors of discrimination which explained 43.7% of the variance. The factors forming the second principal component at Someshwara were the quality of seston, associated with the variations in river run-off and precipitation.

At Surathkal, the magnitude of variations in physico-chemical parameters was secondary (PC2; 21.2% variance) when compared to Someshwara, mainly on reason that it is located farther from the river mouth. However, the most

significant elements forming the first principal component at Surathkal was seasonal variations in seston quantity demonstrated by 37.3% of the variance and high positive loading in SPM. Although a similar variation in seston quantity was observed at Someshwara (PC3), it explained only 12% of the total variance in environmental parameters. The fourth axis ordinates the environmental parameters according to the phytoplankton bloom along with the consequent increase in the Chl-a/POM ratio in the mussel beds.

At Someshwara the variability in temperature, salinity, pH and dissolved oxygen associated with increased river inflow were apparently more important, compared to Surathkal. Average annual discharge from Nethravati and Gurpur Rivers draining to Someshwara is estimated at 15,349 mcm and of Pavanje and Sambhavi rivers draining north of Surathkal is estimated at 1,872 mcm. The freshwater inflows in huge quantities at Someshwara [~9,310 cubic m/sec (ENVIS, 2002)] alter the inshore salinity and temperature and as a consequence negatively impact the mussel populations by influencing their growth, survival and physiological energetics, whereas, the mussel beds at Surathkal are less influenced by such sudden variations. Therefore, with respect to the first two principal components, the two mussel beds were distinctly different with different habitat characteristics.

3.5.4. Condition index and percentage edibility

Condition Index of the mussels of Someshwara and Surathkal presented significant differences, where Surathkal was characterised by high condition relative to Someshwara, but the seasonal pattern of changes in both the beds were similar. The CI ranged from 56 in July to 124 in March at Someshwara and from 41 in June to 186 in March at Surathkal.

In general, mussel condition followed the reproductive cycle, with peak spawning in August-October and a minor in March-April. Subsequent to a peak CI in March, a decrease was observed from May due to spawning, which improved later in August. Ajithakumar (1984) while studying the seasonal variation in the whole body dry weight of *P. viridis* (45-60 mm size), from natural beds off Vizhinjam and Elathur, noticed values ranging from 0.44 g in January (post-spawning) to 1.104 g in August (active gametogenesis) followed by a drop in October subsequent to spawning. At Elathur, the major spawning season was observed during September-November and a secondary spawning season during January-March.

A perusal of literature on dry weight CI shows that there is no information available on the CI of mussels from natural beds off Dakshina Kannada coast for comparison. Further, comparison of CI values in its absolute terms between different periods was found irrelevant due to the differences in the methods of determination. Many workers applied different methods using wet tissue weight or volume in relation to shell cavity volume or weight.

Wet meat percentage or percentage edibility also followed similar trends as CI, with highest meat percentage in September and March prior to spawning, which declined sharply subsequent to spawning. The range of wet meat percentage observed in the study was comparable with the observations of Narasimham (1980) from natural beds and with the observations of Rivonker *et al.* (1993); Mohamed *et al.* (1998) and Rajagopal (1998b) from suspended culture of *P. viridis* along the Indian coast.

There was significant spatial variation in the absolute levels of CI between the two mussel beds, where Surathkal (123.21±49.82) was characterised by high condition relative to Someshwara (94.06±36.31). This emphasises site specific variation in factors controlling mussel condition. CI was higher in pre-monsoon at Someshwara whereas, at Surathkal better condition was observed during the pre-monsoon as well as post-monsoon seasons. Similarly the percentage edibility of the mussels at Someshwara appeared to be slightly lower than that of Surathkal during the study.

3.5.5. Influence of environmental parameters on condition index

Results of PCA identified significant spatial differences between mussel beds in terms of their physico-chemical characteristics and quantity and quality of the seston. Mussels from Someshwara beds generally showed low condition when compared to Surathkal mussel beds that yielded mussels with high condition.

Though considerable seasonal differences were observed in seston content and productivity, no significant difference in these parameters was noticed between the two beds. Multiple regression analysis carried out for each mussel bed independently and in combination provided identical results indicating significant relationships between the water temperature and seston content (POM and chl-a) on the mussel condition. Thus, the measures of these parameters were good predictors of mussel CI in the mussel beds. Water temperature was the most significant variable in the regression equation followed by seston content. The seston content (POM and chl-a) of mussel beds along with water temperature explained 56% of the variations in CI.

Discriminant analysis discriminated the condition profiles in mussels having high CI ratio (CI_{high}) and low CI ratio (CI_{low}) on the basis of chl-a and temperature. The low overall differentiation and segregation with respect to CI_{low} obtained in discriminant analysis support that the reduction in CI by spawning or weight loss even in favourable ecological (food and temperature) conditions.

Temperature along with food availability displayed the strongest positive influence on mussel condition. In the mussel bed, changes in temperature and food availability are mainly associated with monsoon and upwelling. In general, availability of adequate particulate food has been observed as a consistent

stimulus for good growth or condition in bivalves at ambient conditions of water temperature, current speed, water depth and salinity, which influence both bivalve physiology and food availability (Hickman *et al.*, 1991; Thorarinsdottir, 1994; Stirling and Okumus, 1995; Sara and Mazzola, 1997 and Saxby, 2002).

Mussels being poikilothermic, their metabolic rates vary with the ambient water temperature. Therefore, a variation in water temperature certainly plays an important role in the growth of marine mussels through feeding activity and physiological energetics (Widdows *et al.*, 1979; Incze *et al.*, 1980; Page and Hubbard, 1987; Widdows, 1991 and Denis *et al.*, 1999). In tropical conditions temperature is generally at the optimum levels for the bivalves (Alagarswami, 1991) with minor seasonal variations. In the present study, relatively higher water temperature prevailed during October-November and April-May in the mussel beds with a difference of 6°C between the maximum and minimum values. It was observed that the CI as well as the percentage edibility corresponded with these seasonal patterns in temperature, with poor mussel condition in monsoon.

Poor condition during monsoon following the peak in pre-monsoon can be due to the physiological stress induced by unfavourable environmental conditions in the mussel beds. Increased river-inflow and resulting reduction in temperature and salinity can create temporary physiological stress to the mussels. In tropical waters, though mussels are exposed to thermally stable but hotter environment the changes in temperature can affect their growth rate (Chatterji *et al.*, 1984 and Vakily, 1992) in adult mussels as well as development, growth, survival and settlement in spat (Nair and Appukuttan, 2003).

The upper and lower limits of temperature recorded from the mussel beds along the study area were within the tolerable limits for the species and the variations occurred gradually, providing time for gradual acclimation. The optimum temperature for normal growth in green mussels is between 26 and 32°C (Sivalingam, 1977). During thermally unstable environmental conditions, mussels require compensatory acclimation for overcoming stress in order to maintain homeostasis. In marine mussels, similar positive correlations of physiological functions with seasonal variations in temperature have been reported by Bayne and Newell, 1983; Hawkins and Bayne, 1992.

The role of temperature in controlling the cycles of reproductive activity and energy storage which largely influence the changes in CI is widely recognised in mussels (Rosenberg and Loo, 1983). Seed (1976) indicates that temperature is a principal factor in controlling the broader aspects of the annual cycle of mussels. In the present study, consequent to the decrease in rainfall, there was gradual increase in temperature as well as salinity. During this period, the mussel condition improved with tissue build up and gonadal development attaining peak levels in September, followed by the loss in condition due to spawning. Many workers have attempted to explain the effect of environmental parameters on

gonadal development and spawning in bivalve molluscs. Some of these studies laid greater importance on temperature (Newell *et al.*, 1982 and Gaspar and Monteiro, 1999), whereas others focused on food availability (Emmett *et al.*, 1987 and Jaramillo and Navarro, 1995). However, temperature and availability of food have been demonstrated as important decisive factors for somatic growth and gonadal development in bivalves (Seed and Suchanek, 1992; Pazos *et al.*, 1997 and Ceballos *et al.*, 2000), which also appear to determine the duration of the different phases of gametogenesis (Hilbish and Zimmermann, 1988) and spawning (Pazos *et al.*, 1997). Annual reproductive cycle of *P. viridis* along the Indian coast has a direct bearing on the degrees of fatness or condition, which has been related to environmental factors such as temperature, salinity and food availability (Rao *et al.*, 1975; Nagabhushanam and Mane, 1975; Qasim *et al.*, 1977; Ajithakumar, 1984; Parulekar *et al.*, 1982; Rivonker *et al.*, 1993 and Rajagopal *et al.*, 1998a).

Along the east coast, peak reproductive activity of *P. viridis* coincided with rising water temperature (Rajagopal *et al.*, 1998b) as well as with increase in salinity (Narasimham, 1980). Reproductive activity of *P. viridis* along the west coast of India was related to increasing salinity (Nagabhushanam and Mane, 1975); increasing salinity and phytoplankton (Ajithakumar, 1984) and increasing salinity, temperature and abundance of food material (Parulekar *et al.*, 1982).

Even though the CI follows a reproductive cycle controlled by complex balance between exogenous factors and endogenous factors (Seed, 1976; Kautsky, 1982 and Rajagopal, 2006), the timing of the various components of the reproductive cycle was found to vary depending on the mussel population and water temperature. Therefore, reproductive activity of *P. viridis* can vary substantially based on the environmental conditions within narrow geographical regions, even in closely located places on a given coast. This could probably explain the differences in absolute levels of accretion in somatic /gonadal tissue or increase in CI of *P. viridis* at these sites (Rajagopal *et al.*, 2006). Obviously, local hydrographical conditions and food availability may act as primary governing factors that influence the timing of spawning by a population (Lee, 1985; Newell *et al.*, 1982 and Rajagopal, 2006).

Another important factor influencing CI of *P. viridis* observed in the study was food availability. Mussels feed mainly on unicellular algae (Bayne and Hawkins, 1992) and to a lesser extent on detrital material (Rodhouse *et al.*, 1984 and Langdon and Newell, 1990) albeit the latter is reported to be a poor quality food (Williams, 1981 and Ruckelshaus *et al.*, 1993). Chlorophyll-a content of the mussel beds observed was generally high through out the year, except during the early monsoon. It was also noticed that during early monsoon (June-July) there was an increase in the seston content (SPM, POM and PIM). The elevated POM levels and the low chl-a/POM ratio indicated changes in the quality of the seston in terms of its food value. Previous works indicate that mussels can select organic over inorganic particles and can even discriminate among organically-

rich particles over less desirable food items, thereby selectively ingesting more nutritive particles and rejecting less nutritious phytoplankton, detrital and silt particles as pseudofaeces (Bayne *et al.*, 1989 and Hawkins *et al.*, 1998). This strongly affirms that the mussels have the ability to alter their feeding behaviour in response to the available food and enable them to obtain maximum benefit from the food supply (Griffiths, 1980; Bayne *et al.*, 1989; Bayne and Hawkins, 1992; Navarro *et al.*, 1991 and Hawkins *et al.*, 1998). However, in the present study, from the decrease in CI of mussels despite abundant seston, it appears that when there is resuspension of particulate organic matter in the ecosystem, the mussel bed acted as a selective filter for only phytoplankton (Prins *et al.*, 1996) or the physiological stress due to variations in physico-chemical parameters along with the high seston loads (56-114 mg/l) reduced particle selection (Urban and Kirchman, 1992). Barille *et al.* (1997) observed a decrease in the scope for growth at high seston concentrations (> 160 mg/l) which they attributed to decreased selectivity due to an overloading of the ctenidia and/or the labial palps.

The low chl-a/POM ratio indicates a poor quality of organic seston and a high detritus fraction which entered the system in relation to rains and water discharge (Garen *et al.*, 2004). The resuspension associated with monsoon, in effect replaced the phytoplankton content in the seston with the detritus. The suspended detritus containing organic matter in general is nutritionally poorer than the micro algae. In addition, reduced temperature with monsoon, along with a variety of other stressors, such as decreased salinity or increased turbidity could have compounded the effects of decreased phytoplankton concentration on mussel health.

With the receding southwest monsoon, the chl-a content registered an increasing trend and consequently, the mussel condition improved during late monsoon. In many bivalves, it is observed that when favourable conditions prevail following a period of stress they are able to reactivate tissue accretion by utilizing the abundant food available in their beds. When food supplies were adequate, increased water temperature was associated with improved growth and condition in bivalves (Seed and Suchanek, 1992 and Saxby, 2002).

In the present study improvement in CI was observed during September–October when, the physico-chemical condition became favourable after monsoon. When food is abundant, surplus energy is used by bivalves for growth of somatic tissues and also for gonadal development (Urrutia *et al.*, 1999 and Ojea, 2004). Myint and Tyler (1982) and Walter (1982) correlated reproductive cycles in *P. viridis* with availability of food resources and salinity.

In the mussel beds the peak chl-a levels observed in March corresponded with peak in CI. Among sessile suspension feeders, seston availability is generally, the single most important exogenous variable acting to regulate condition (Bayne and Worall, 1980; Rodhouse *et al.*, 1984; Smaal *et al.*, 1986; Bayne *et*

al., 1987 and Prins *et al.*, 1998). Over longer time scales, a number of researchers have observed differences in the growth of bivalves that were related to seasonal cycles in primary production (Vahl, 1980; Toro, 1995; Toro *et al.*, 1999; Sara and Mazzola, 1997; Sara *et al.*, 1998).

The aim of this study was to employ the relative changes in CI of green mussels as physiological stress indicators for the evaluation of the overall health of the two mussel beds along the Dakshina Kannada coast. Biomarkers are useful tools for understanding the complex interactions that govern organism's responses to environmental stressors and their sub-lethal effects on its health. The study indicated that there is obvious seasonal variation in the mussel condition with a general trend towards lower values in the monsoon. Besides the seasonal variability, differences among the mussel beds investigated were also observed. Higher condition indices were recorded in mussels collected at Surathkal, whereas, the condition index recorded at Someshwara was always lower than Surathkal regardless of the sampling season.

Analysis of the factors influencing the CI revealed that in the seawater of Someshwara and Surathkal, the availability of seston in quantitative terms was high throughout the period of study. Hence it can be presumed that food availability has not acted as a limiting factor in controlling the biological activity of the mussel. Conversely, qualitative changes in food availability due to compensation of phytoplankton by detrital organic matter emerged as a limiting factor. In general, there was spatial variation in the quality of food available for the mussels and increase in CI was associated with increased abundance of phytoplankton and increasing temperature.

The physico-chemical parameters of mussel bed off Surathkal offer a more conducive environment for the growth of mussels, characterised by better CI with low seasonal variations. The comparatively low CI in Someshwara mussel bed can be attributed to wider fluctuations in environmental parameters associated with monsoon. Under fluctuating environmental conditions associated with monsoon, CI became more affected by temperature variations than by availability of food. Consequently at ambient water temperature, increased food availability was signified by improved growth and condition.

Freshwater inflows to the coastal waters result in significant changes of physico-chemical parameters which affects the structure and function of ecosystems. The synergistic influence of temperature and seston along with the other environmental variables affect virtually all aspects of the biology of marine mussel. Though there are many studies reporting the responses of *P. viridis* to temperature and food availability, the responses are likely to vary based on the variations in the synergy of parameters. Thus, site-specific assessments are important in analysing the cause-effect relationships.

The study has helped in understanding the response of the local mussel population to natural stress factors. The environmental data presented different

degrees of temporal variability in the mussel beds and yielded biological responses as indicative of multiple stressors on the mussel. Reserves were alternatively channelled into energy-consuming processes and mussels during unfavourable environmental conditions showed poor tissue condition as they deplete energy reserves which were potentially destined for growth. Thus it can be concluded that condition index, besides its representation of the general health condition of the bivalves can potentially be used as an integrated measure of physiological stress experienced by the organism.

Chapter 4

Chemical contaminants in shellfish waters and biomarker response in mussels

4.1. Introduction

Environmental pollution is becoming an area of mounting concern in recent years since urbanization, industrialization and other developmental activities are exerting tremendous stress on the coastal ecosystems. Chemical contaminants such as trace metals, chlorinated hydrocarbons and polycyclic aromatic hydrocarbons are known to be widespread in the marine ecosystem (Kadhim, 1990). The biota of the coastal environments is continuously challenged by the mounting load of pollutants and many aquatic organisms like marine mussels have the potential to accumulate these contaminants in their soft tissues. Hence, it is necessary to monitor the levels of various contaminants in the coastal environments to protect the fragile ecosystem as well as to ensure that the quality of the fish or shellfish harvested from such waters is safe for consumption.

In coastal waters, inorganic contaminants such as trace metals are derived from a variety of natural and anthropogenic sources. Distribution of trace metals in the soft tissues of mussels have been extensively studied, from both ecotoxicological and seafood safety points of view in several countries (O'Connor, 1992; Krishnakumar *et al.,* 1990 a,b, 1994, 1998; Szefer *et al.,* 2004 and Glynn *et al.,* 2003). Although trace metals in low concentrations are normal constituents of

marine organisms, at high levels they are potentially toxic and may disrupt the biological activities of aquatic organisms.

Dakshina Kannada District of Karnataka is among the fast growing industrial centers of the State. As a consequence, industrial effluents are discharged into the coastal environment. Apart from the industrial effluents, the inshore coastal area receives sizeable volume of runoff from agricultural areas and nearby settlements. Consequently, contamination of marine ecosystems along the Dakshina Karnataka coast with trace metals and xenobiotics receives continued attention in the scientific literature (Krishnakumar *et al.*, 1990a,b, 1998, 2004b; Mohankumar *et al.*, 2003; Sasikumar *et al.*, 2006 and Verlecar, 2006) and environmental monitoring programmes (CMFRI, Annual Reports, 2002). These investigations have documented levels of contaminants in the seawater, sediments, bivalves and submerged aquatic vegetation.

The monitoring programmes are currently focused on analysing the levels of specific chemicals released into the aquatic environment and thereby provide useful information on the levels of contamination. But these studies generally do not provide information on the effects of the contaminants on biological systems. Therefore, recent programmes are more oriented towards effects (biologically-based) monitoring, relying on biomarkers of environmental contaminants (Krishnakumar *et al.*, 2004b) than contaminant (chemically-based) monitoring. Marine mussels are by far, the most popular sentinel organism used in pollution monitoring studies, because of their sessile nature, availability, mode of feeding, bioaccumulation abilities and likelihood of human consumption. Due to their feeding nature they can accumulate chemical contaminants such as trace metals and persistent organic pollutants (POPs) present in the habitat.

The application of biomarkers is considered as a feasible and cost-effective approach in pollution studies, since monitoring of all chemicals present in the environment is practically impossible. Additionally, routine measurements of all possible contaminants in environmental compartments, such as the water-column, are difficult as concentrations are generally below the detection limits and analysis, require sophisticated and expensive techniques. Further, monitoring the concentration of specific pollutants alone does not reveal their impact on the living organisms, especially the synergistic effects in combination with the intrinsic or extrinsic factors. Many contaminants, especially POPs can cause significant biological changes at very low environmental concentrations but the harmful effects of these chemicals on living organisms remain largely unknown.

Generally, biological changes in any organism resulting from contaminant exposure are initiated at the cellular and molecular levels. Many chemical, physical and environmental stressors including pollutants are known to destabilize lysosomal membranes in molluscs and hence several studies have used lysosomal membrane stability as a reliable indicator of stress imposed by

environmental pollutants (Moore and Lowe, 1985). The term "biomarker or biomarker response" describes the sub-organismic changes, such as those occurring at cellular, biochemical, molecular or physiological levels, that can be measured in cells, body fluids, tissues or organs within an organism and are indicative of xenobiotic exposure and/or effect (Lam and Wu, 2003).

In this context, the present study was carried out to assess the levels of trace metals and organochlorine pesticides in seawater, their bioaccumulation in the soft tissues of green mussels and the subsequent biological effects (lysosomal membrane stability) of environmental pollution if any, on the health of mussels.

4.2. Review of Literature

4.2.1. Trace metals

The capacity of mussels to accumulate potentially toxic trace metals in their tissues far in excess of environmental levels is well documented (Phillips, 1977 and Rainbow, 1995). Hence, mussels are studied extensively as a potential biomonitor in ecotoxicological studies through out the world (Davies and Pirie, 1980; Martin, 1985; Classie, 1989 and O'Connor, 1996). Biomonitors are generally used to establish geographical and/or temporal variations in bioavailability of trace metals in marine environment, offering time-integrated measures of those portions of the total ambient metal load that are of direct ecotoxicological relevance (Rainbow, 1995). Studies on the levels of contaminants in mussels have also been conducted to check contamination levels in the interests of public safety (Tan and Lim, 1984; Phillips and Muttarasin, 1985 and Yap *et al.*, 2004a). Species of the genus *Perna*, *P. perna* and *P. viridis* have tremendous potential as trace metal biomonitors, particularly in warmer waters (Rainbow, 1995) and have been used to assess the state of marine pollution by toxic contaminants and to understand the fate and effects of contaminants in the Southeast Asia-Pacific region (Tanabe, 1994).

A perusal of literature revealed that significant progress has been made in many regions of the world in assessing the residual levels of trace metals in mussel tissue, the spatial and temporal trends in trace metal contamination, factors affecting bioaccumulation and their effects on physiological responses of mussels.

The primary objective of assessing the levels of chemical contaminants in coastal waters is to protect public health and the valuable living natural resources (Widdows and Donkin, 1992). Tan and Lim (1984) reported linear relationship in the uptake of lead by green mussel *P. viridis* with time, emphasizing the need to monitor the trace metal levels in bivalves to ensure that they are safe for human consumption. Similarly, Phillips and Muttarasin (1985) analysed the levels of trace metals in green mussel *P. viridis*, along with clam *Paphia undulata*, cockle *Anadara granosa* and oyster *Crassostrea commercialis* for evaluating the public health hazard in Thailand. Trace metal accumulation by *P. viridis* was monitored along the coastal areas of Penang by Sivalingam (1984) to find out the contamination levels of Pb and Zn. Phillips (1985) studied the trace metal bioaccumulation in *P. viridis* from Hong Kong waters. Luis *et al.* (1989) measured the seasonal variations in levels of Cd, Hg and Pb in *Corbicula manilensis, P. viridis, Ostrea malabonensis and Arca* spp. from Philippines to demonstrate that the bivalves are safe for human consumption. Chan (1989) studied the spatial and temporal variations in tissue concentrations of Cd, Cu, Pb and Zn in *P. viridis* transplanted to four experimental sites in Hong Kong.

Burns (1990) determined the trace metal levels in *P. viridis, Mytilus edulis* and *Arca zebra* from two Bermudan harbours for comparing point and non-point source of contamination. Concentrations of Fe, Cu, Zn, Cd and Pb were determined by Chu *et al.* (1990) in samples of sediment, mussel *P. viridis* and rock oyster *Saccostrea cucullata* from nine locations in Tolo Harbour, Hong Kong. Concentrations of Zn, Mn, Cu, Cr, Ni and Cd were determined in the whole soft parts of the mussel *P. viridis* by Sukasem and Tabucanon (1993) along the Gulf of Thailand and compared with the earlier accumulation trends. Sze and Lee (1995) measured the mucus secretion rate in *P. viridis* and *Septifer virgatus* when chronically exposed to Cu. Cheung *et al.* (1998) studied the tissue metal concentrations and condition indices in *P. viridis* from five stations along Tolo Harbour, Hong Kong. Rees *et al.* (1999) evaluated the impact of land-based contaminants on benthic fauna in Jakarta Bay, Indonesia by measuring dissolved concentrations of Pb, Cu, Zn, Cr and Ni in seawater, in suspended particulate matter, sediments and in corals as well as green mussel *P. viridis* and reported that the concentration of metals in water is the primary route for metal uptake by the corals and mussels.

Seasonal variations of metal accumulation in mussel *P. viridis* from Hong Kong waters were investigated by Wong *et al.* (2000) to find out the risk in consuming the mussels. Chiu *et al.* (2000) reported the concentrations of trace metals in *P. viridis* from three mariculture zones of Hong Kong and compared with earlier trace metal monitoring results. Chong and Wang (2000) analysed the influence of the metal concentration in the sediment, the presence of phytoplankton and the oxidation condition of the sediment on the degree to which sediment bound Cd, Cr and Zn were assimilated by *P. viridis* and the Manila clam *Ruditapes philippinarum*. Seasonal and spatial trends in trace metal contents in 30 species of molluscs, including *P. viridis* were reported by Hung *et al.* (2001) as part of a long-term programme of Asia-Pacific Mussel Watch from Taiwan.

Chong and Wang (2001) employed a kinetic approach to determine the rate of metal uptake (Cd, Cr and Zn) from the dissolved phase and the rate constants of metal depuration in the mussel *P. viridis* and the clam *Ruditapes philippinarum*. Yap *et al.* (2002) reported the concentrations and speciation of Cd, Cu, Pb and Zn in surface sediments from west coast of Peninsular Malaysia and correlated with the levels in the soft tissue of *P. viridis*. Blackmore and Wang (2003) studied trace metal (Cd, Cr, Se and Zn) uptake from the dissolved phase, assimilation efficiency from the dietary phase and metal body burden as well as the clearance rate in green mussel.

Wang and Wong (2003) examined the assimilation of Cd, Cr and Zn by the green mussel *P. viridis* under combinations of different compositions and concentrations of food (diatom and sediment) and variable food quantity and quality during particle digestion. The distributions of Cd, Pb and Zn in the total soft tissues, byssus and total shells of *P. viridis* were studied in field collected samples as well as in laboratory experimental samples from the west coast of

Peninsular Malaysia by Yap *et al.* (2003a,b). The results indicated that Cd, Pb and Zn were readily accumulated in the whole shells. Nicholson and Szefer (2003) reported the accumulation of metals in the soft tissues, byssus and shell of *P. viridis* from polluted and uncontaminated locations in Hong Kong coastal waters. Fung *et al.* (2004) indicated higher contaminant levels including trace metals in *P. viridis* and blue mussel *Mytilus edulis* collected from seven locations along the east coast of China. Yap *et al.* (2004a) determined the trace metal levels in *P. viridis* from the west coast of Peninsular Malaysia and reported that the trace metal levels were lower than the permissible limits for human consumption. Toxicities and tolerances of Cd, Cu, Pb and Zn in *Isochrysis galbana* and in *P. viridis* were conducted by short-term bioassays using endpoints, growth, production and mortality by Yap *et al.* (2004b). They reported that the LC_{50} values for the mussels were 1.53 mg/l for Cd, 0.25 mg/l for Cu, 4.12 mg/l for Pb and 3.20 mg/l for Zn. Green mussel was found to be most sensitive to Cu, followed by Cd, Zn and Pb. Cross-flow ultrafiltration and radiotracer techniques were used to study the influences of natural dissolved organic carbon (DOC) and colloidal organic carbon (COC) on the bioavailability of Ag, Cd and Cr to the green mussel *P. viridis* by Pan and Wang (2004). Shi and Wang (2004) quantified the accumulation of Cd, Hg, Ag and Zn in the green mussel *P. viridis* affected by previous exposure to Cu, Ag or Zn. Yap *et al.* (2006) studied the bioavailability and tissue redistribution of Cu and Pb in *P. viridis.*

In Indian coastal waters Zingde *et al.* (1976) studied the concentration of trace metals in *Saccostrea cucullata* from Goa waters. Sankaranarayana *et al.* (1978) explored the seasonal variations in metal concentration in *Crassostrea madrasensis* from Cochin region. Kureishy (1981; 1983) reported the concentrations of some essential and non-essential trace elements in zooplankton, crustaceans, bivalves and fishes of the Andaman Sea. Patel and Chandy (1988) reported seasonal distribution of mercury in the soft tissues of *Anadara granosa* from Thane Creek. Rajan *et al.* (1991) evaluated the effect of seasonal variations in the Zn concentrations in *Meretrix casta.* Seasonal variation of copper accumulation in *Marcia recens* was reported by Muralidharan and Raje (1997). Senthilnathan and Balasubramanian (1998) reported trace metal concentration in oyster *C. madrasensis* from the estuaries of southeast coast of India.

Along Indian coast, seasonal distribution of trace metals in *P. viridis* was reported by Lakshmanan and Nambisan (1983). At Calicut and Munambam area along the southwest coast of India, levels of selected trace metals in marine mussel *P. viridis* were investigated by Radhakrishnan (1994). Pillai *et al.* (1986) studied the levels of Fe, Zn, Cu, Pb, Cd, Ni and Co in *P. viridis* from Calicut. The trace metal residues in fish and shellfish were analysed by Sankar *et al.* (2006) from the Calicut area to evaluate the status of the chemical contaminants in seafood.

Senthilnathan *et al.* (1998) reported the seasonal variation of metal levels in *P. viridis* and *C. madrasensis* at four stations along the southeast coast of India. Rivonker and Parulekar (1998) studied the accumulation of trace metal in *P. viridis* off Goa. Tewari *et al.* (2000, 2001) analysed the trace metal levels in *P. viridis* from natural mussel beds off Mocha, Gujarat and found a linear relationship with mussel length.

Evidence of trace metal accumulation in the soft tissue of green mussel was documented from coastal waters adjacent to the urbanized areas of Karnataka. Krishnakumar *et al.* (1990a) determined the trace metal concentration in *P. viridis*, oyster *C. madrasensis* and seaweed *Sargassum* sp. from the vicinity of discharge point of a caustic soda plant from Karwar and reported that the mussels are effective biomonitors for Pb and Mn. The analysis of bioaccumulation of trace metals in different biota revealed comparatively higher levels in the oyster *S. cucullata* and green mussel *P. viridis.* Investigation on the bioaccumulation of mercury in *P. viridis* was carried out from the same area by Krishnakumar and Pillai (1990). Krishnakumar *et al.* (1990b) measured the physiological and cellular responses of *P. viridis* on exposure to Cu and Hg and found significant decrease in O:N ratio, scope for growth and growth efficiency in comparison to controls. Krishnakumar *et al.* (1998) while assessing the distribution of trace metal in the biotic and abiotic matrices along Karnataka coast reported the levels of trace metals in *P. viridis.* Sasikumar *et al.* (2006) studied the spatial trends in trace metal bioaccumulation in *P. viridis* collected from 28 sampling sites along Karnataka coast in 2002.

Studies on the trace metal levels in sea water along southwest coast of India are limited to few locations. Sengupta *et al.* (1978) and Qasim and Sengupta (1981) monitored the trace metals in seawater of Arabian Sea. Rivonker and Parulekar (1998) studied the seasonal variation of trace metals in seawater in the Dona Paula Bay, Goa. Other important works on the trace metal levels in seawater along coastal waters of Karnataka coast are those of Krishnakumar *et al.* (1998, 2004a).

In the present study, the levels of trace metals Cd, Pb, Cu, Zn, Ni and Fe in the coastal waters were analysed and their presence and magnitude in the mussel tissue were studied to investigate the pattern of bioaccumulation.

4.2.2. Organochlorine pesticides

Persistent organic pollutants (POPs) are a group of chemicals that are resistant to natural degradation processes and are therefore extremely stable and long-lived. They are synthetic chemicals released to the environment by anthropogenic activities and many of them are highly toxic and have the potential of bioaccumulation in the biota. In recent decades, numerous POPs have been produced worldwide in large scale and many are still in production and use. Though a few of them notably organochlorine pesticides (OCPs) have been

banned in developed countries, due to their persistence, high levels of such contaminants are still found in natural water bodies. In addition, many OCPs are still being used in agriculture and vector control programmes in many parts of the world. Being lipophilic in nature, OCPs tend to get accumulated in the lipid fractions and thus get magnified in organisms higher in the food chain. A number of studies of organochlorinated compounds in the marine biota, seawater and sediment have been undertaken through out the world, but only a few studies have been carried out in India.

Dichloro-diphenyl-trichloroethane (DDT) is one of the best known synthetic pesticides that is banned in many countries for agricultural use but its use in vector control continues in many parts of the world. The DDT is essentially a mixture of two isomeric compounds comprising 65 to 70% of para, para'-DDT (*p,p'*-DDT) and 30 to 35% of ortho, para'-DDT (*o,p'*-DDT). Based on the studies in bivalves, the estimated half-life of DDT is 10-20 years and in the process gets degraded into DDE (Dichloro-diphenyl-dichloroethylene) and DDD (Dichloro-diphenyl-dichloroethane), the two major persistent DDT metabolites. DDE is formed by the loss of hydrogen chloride (dehydrohalogenation) of DDT and is one of the more common breakdown products. DDE, like DDT is fat soluble and hence tends to build up in the fat of animals. Due to its stability in fat, DDE is rarely excreted from the body and body levels tend to increase throughout life. DDE is reputed to be more toxic compared to DDT, hence is not desirable to accumulate in soil. DDD is similar in properties to DDT, but it is considered to be less toxic to animals than DDT.

Hexachlorocyclohexane (HCH) also called benzenehexachloride (BHC) is a mixture of the α, β, γ, δ and ε isomers, which are chemically stable compounds and differ regarding their persistence in soil, water and living organisms. Studies conducted on the persistent organochlorine compounds identified DDT and α-BHC as the predominant compound in green mussels from selected locations along the Indian coast (Monirith *et al.*, 2003).

Heptachlor is a non-systemic insecticide widely used as an agricultural and domestic pesticide, the principal metabolite of this is heptachlor epoxide. Aldrin is yet another widely used pesticide for the control of pests in soil and it degrades to dieldrin, a persistent insecticide. Endrin is the stereo-isomer of dieldrin more active than dieldrin and is used as an insecticide and rodenticide.

Green mussels with a wide geographical distribution in the Southeast Asia-Pacific region are grown as commercially valuable seafood for worldwide markets. However, only a few studies have been conducted in the region on the levels of OCPs and other contaminants in green mussel (Prudente *et al.*, 1999; Monirith *et al.*, 2003; Kan-atireklap *et al.*, 1997 and Ramesh *et al.*, 1990). Kannan *et al.* (1995) reported the presence of organochlorines in muscle tissue of marine fish collected during 1989-1993 in eastern and southern Southeast Asia (India, Thailand, Vietnam, Indonesia, Papua New Guinea, the Solomon

Islands and Australia). Monirith *et al.* (2003) analysed the levels of OCPs in green mussels from the coastal waters of Asian countries including 17 locations along the Indian coasts.

While a number of studies in many countries have demonstrated the contamination with organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) in green mussels (Phillips, 1985 and Monirith *et al.*, 2003), information on the concentrations of pesticides in the coastal environment and mussel beds of Indian waters is inadequate. Published scientific literature on levels of OCPs in green mussel from India is limited to the studies by Radhakrishnan *et al.* (1986, 1994) and Ramesh *et al.* (1990) from coastal waters of south India. A recent study reported concentrations of chemical contaminants including organochlorine residue in fish and shellfish along the southwest coast of India (Sankar *et al.*, 2006).

Few studies have reported the distribution of OCPs in seawater of the central west coast of India (Sarkar and Sengupta, 1989 and Pandit *et al.*, 2006). Similar to the investigations on the organochlorine levels in mussel tissue, levels in environmental compartments such as seawater and sediment from the mussel beds of India have not received any attention till date.

4.2.3. Lysosomal membrane stability

Chemical contaminants in the environment have been found to exert inhibitory effects on several physiological processes as well as affect the functions of cellular constituents, including lysosomes in bivalves (Moore, 1980, 1984; Pickwell and Steinert, 1984; Suresh and Mohandas, 1990; Krishnakumar *et al.*, 1994, 2006; Lowe and Pipe, 1994; Lowe *et al.*, 1995b; Cheung *et al.*, 1998 and Francioni *et al.*, 2007). Lysozyme in bivalves is involved with the mechanisms of internal host defense and digestion (Cheng, 1983a,b). Many xenobiotics induce alterations in the bounding membrane of the lysosomes leading to destabilization (Moore and Lowe, 1985). Lysosomes also play a terminal role in the cellular homeostasis of metals through sequestration and storage of metal-binding metallothioneins (Bayne, 1989). Lysosomes have been found to be an ideal starting point for investigations of generalised cell injury in marine molluscs as many cell types in these animals are particularly rich in lysosomes (Summer, 1969). In lysosomes, membrane destabilisation or increased permeability results in reduced latency of the enzymes (Moore and Clarke, 1982) leading to the release of degradative hydrolytic enzymes from the lysosomal compartment into the cytosol (Moore, 1976). Studies by Moore *et al.* (1996a) demonstrated that increased fragility of the lysosomes derived from autophagic breakdown of intracellular proteins, leads to shorter retention times for the neutral red applied to haemocytes.

Many researchers have used lysosomal membrane stability in molluscs as a reliable indicator of stress imposed by environmental pollutants. Lowe and Pipe

(1994) measured damages to lysosomes in isolated molluscan digestive cells *in vitro* following *in vivo* exposure of mussels to polycyclic aromatic hydrocarbon, fluoranthene for 7 days. The study indicated impairment of functional integrity of the lysosomal membrane following hydrocarbon exposure illustrating that lysosomes are the target of toxic action of pollutants. Lowe *et al.* (1995b) compared neutral red retention in haemolymph and latency for digestive cell lysosomes, following *in vivo* fluoranthene exposure in *M. edulis* and indicated that there was good agreement between the tests in terms of their ability to demonstrate a detrimental contaminant effect. Cajaraville *et al.* (1996) studied in *vitro* activities including neutral red uptake assay in mussel haemocytes (*M. galloprovincialis*) as biomarkers of environmental quality in coastal and estuarine areas.

Grundy *et al.* (1996) examined the phagocytosis and lysosomal Neutral Red Retention Assays (NRRA) in haemolymph in *M. edulis* by *in vivo* exposure experiments to examine the effects of PAHs on particle uptake by haemocytes and lysosome integrity. Moore *et al.* (1996b) investigated the lysosomal response within molluscan haemocytes isolated from mussels, as a biomarker of pollutant exposure to a number of hydrocarbons using NRRA demonstrating the use of this non-destructive biomarker technique to detect pollution gradients *in situ*. Moore *et al.* (1997) evaluated the fate and potential toxicological effects of non-calorific synthetic fat sucrose polyester (SPE) in aquatic systems based on the lysosomal NRRA. Cheung *et al.* (1998) studied the sub-cellular perturbations in the lysosomal compartment of haemocytes in *P. viridis* among indigenous populations along Tolo Harbour, Hong Kong using NRRA technique. Nasci *et al.* (1998) studied the neutral red retention time of mussel, *M. galloprovincialis* from the polluted area of the Venice Lagoon along with the tissue concentration of chemical contaminants (Hg, Pb, Cd, Zn, Cr, DDTs and PCBs) to identify their possible relationships. Camus *et al.* (2000) studied the stability of lysosomal and cell membranes in haemocytes of the mussel *M. edulis* at low temperatures. Fernley *et al.* (2000) inversely correlated the results of NRRA with PAH concentration in mussel haemocytes. Shepard and Bradley (2000) reported increased lysosomal damage in *M. edulis* exposed to graded copper concentrations with each increase in dosage using NRRA. Ros *et al.* (2002) evaluated the potential use of lysosomal destabilisation as a biomarker of anthropogenic stress in *M. galloprovincialis* and indicated that the lysosomal response measured in haemocytes is a more valuable biomarker of anthropogenic stress in marine coastal environments. Marigómez and Villacorta (2003) elucidated the lysosomal size alterations in digestive cells of mussel *M. galloprovincialis* against organic chemical compounds and general stress.

Dailianis *et al.* (2003) applied NRRA in tissues of *M. galloprovincialis*, in pollution monitoring. Brown *et al.* (2004) used multiple biomarkers, molecular, cellular and physiological in blue mussel *M. edulis* to determine the variability of sublethal effects of copper. The study illustrated that the effects at the molecular level can be used to interpret the level of physiological impairment of

the organism. Castro *et al.* (2004) used neutral red retention time measured in mussel haemocytes (*M. galloprovincialis*) gathered from nine different locations reflecting different degrees of anthropogenic contamination, as a potential biomarker of environmental contamination along the Portuguese coast. Harding *et al.* (2004) evaluated NRRA as a stress response indicator in cultivated mussels (*Mytilus* spp.) in relation to post-harvest processing activities and storage conditions. Nesto *et al.* (2004) adopted a multiple biomarker approach for evaluating the stress influencing the biological responses of mussel *M. galloprovincialis* from the Lagoon of Venice, Italy. Mamaca *et al.* (2005) studied the biological effects of styrene in laboratory-exposed mussels (*M. edulis*) by the NRRA on haemolymph.

As a part of the multidisciplinary programme on the biological effects of environmental pollution in marine coastal ecosystems of the European Commission, Auffret *et al.* (2006) validated the immunological alterations in mussels (*M. galloprovincialis*) as biomarkers of exposure to chemical contamination in polluted areas of Western Mediterranean. They suggested alterations in haemocyte counts, lysosomal stability and phagocytosis as the most reliable effects in polluted sites. Nigro *et al.* (2006) investigated genotoxicity and lysosomal alterations in haemocytes by the NRRA in the Mediterranean mussel (*M. galloprovincialis*) from Tuscany, Italy. Okay *et al.* (2006) investigated the toxicity of pyrene to *M. edulis* and *M. galloprovincialis* and correlated the decreasing trend in health status of the mussels with pyrene bioaccumulation, indicated by the NRRA. Canty *et al.* (2007) determined the sublethal impact of short-term exposure to azamethiphos on the blue mussel, *M. edulis* by NRRA. Francioni *et al.* (2007) carried out the bio-effect monitoring of the mussel *P. perna* using lysosomal membrane stability as a biomonitor of polycyclic aromatic hydrocarbon (PAH) exposure.

In India, Suresh and Mohandas (1990) studied the activity patterns of the lysosomal marker enzyme, acid phosphatase, in the haemolymph of clams *Sunetta scripta* and *Villorita cyprinoides* exposed to three sub-lethal concentrations of copper. The results indicated destabilisation of lysosomal membrane with metal exposure. In Karnataka-Kerala coast, investigations by Krishnakumar *et al.* (2004b) evaluated the biological responses such as chromosomal aberration, Sister Chromatid Exchange (SCE), micronuclei formation, hemic neoplasia and DNA damage in green mussels to xenobiotics in the coastal waters of Karnataka.

4.3. Materials and Methods

4.3.1. Sampling and preservation

4.3.1.1. Trace metals

Seawater and mussel samples were collected from Someshwara and Surathkal mussel beds as detailed in Chapter 2 during January 2003-April 2004. The mussels were depurated overnight in filtered seawater and stored frozen until analysis. Seawater samples for trace metal analysis were collected in acid washed polyethelene flasks. Immediately on arrival at the laboratory, the water samples were filtered through a 0.45 mm cellulose acetate filter and the organic matter was digested by adding HNO_3 to a final pH of < 2 and kept frozen for analysis.

4.3.1.2. Organochlorine pesticides

Composite mussel tissue samples collected from the mussel beds during February-March, June-July and September-October were used for pesticide analysis. During each sampling, a sub-sample of 30 mussels was preserved for the analysis of organochlorine pesticides.

Seawater samples collected during June-July and September-October were used for pesticide analysis. The water samples collected were stored in glass bottles, refrigerated at 2±5°C until analysis.

4.3.2. Analytical methods

4.3.2.1. Trace metals in seawater

Trace metals were analysed using Stripping Voltammetry in a 757 VA Computrace attached to 765 Dosimat (Metrohm, Switzerland). A hanging mercury drop electrode was used as the working electrode and potentials were measured against KCl 3 mol/l reference electrode and an auxiliary platinum electrode. Total dissolved Zn, Cd, Pb and Cu were estimated by adding 10 ml sample and 1 ml acetate buffer in a Teflon cell, using Differential Pulse Anodic Stripping Voltammetry (DPASV). The Concentrations of these metals were simultaneously measured by addition of mixed metal standards using Dosimat (Florence, 1972). Total dissolved Ni was estimated using Cathodic Stripping Voltammetry (CSV) by adding 10 ml sample, 0.05 dimethyl glycoxime (DMG) and 0.5 ml NH_3 buffer into the Teflon cell and analysed using the standard (Meyer and Neeb, 1983). The accuracy of the analytical procedure was checked using certified reference material (BCR, CRM 403 Sea-Water). The recovery estimated by measuring standard spiked samples was 97% for all the metals studied.

4.3.2.2. Trace metals in mussel tissue

Mussels (size >50 mm) were rinsed with deionised water and wet shucked. The whole tissue mass from 10 mussels were pooled, homogenized and used for the analysis. Approximately 1-2 g of tissue was digested with 10 ml mixture of HNO_3 and H_2O_2 (1:1 ratio) following standard procedures (Robisch and Clark, 1993). The trace metals in the digested samples were determined by Atomic Absorption Spectrophotometry with either airacetylene flame (zinc) or graphite furnace (copper, cadmium, nickel, lead). The results were expressed in ppm wet tissue weight. Blanks and standards were digested with each sample set to provide quality control. The accuracy of the method was verified (10 replicates) using standard reference material (fish tissue, MA-B3/TM) obtained from the International Laboratory of Marine Radioactivity, IAEA. Recoveries were above 90% for all the trace metals measured.

4.3.2.3. Trace metals in mussel shell

The shells after wet shucking were cleaned and rinsed with deionised water. The periostracum and the calcite section of the recently formed shell layers were scrapped off leaving only the nacreous part of the shell (Bourgoin, 1990). Strips of the recently formed nacreous layer from the shells were used for analysis. Approximately 1-2 g of the sample was digested following standard procedures as detailed above (Robisch and Clark, 1993). The results were expressed in ppm shell weight.

4.3.2.4. Organochlorine pesticides in seawater

Liquid-liquid extraction gas chromatographic method was used for the extraction and analysis of organochlorine compounds. The extraction and cleanup of pesticide residues were carried out following APHA (1998). The pesticides residues in seawater were initially extracted using methylene chloride and then the individual pesticides (Aldrin, α-BHC, β-BHC, γ-BHC, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE *o,p'*-DDT, *p,p'*-DDT dieldrin, endrin, heptachlor, heptachlor epoxide) were measured by gas chromatography using electron capture detector (ECD).

Pesticides were extracted from one litre of water with two successive portions of methylene chloride. The water residues were collected by treatment with anhydrous sodium sulphate, then concentrated on a rotary evaporator under reduced pressure and cleaned-up by elution from a column of silica gel as described in Thompson *et al.* (1977).

Quantification of organochlorines was made on gas chromatograph (Perkin Elmer-Autosystem XL) equipped with ^{63}Ni electron capture detector (ECD) and Elite-5 column (Perkin Elmer, 30 m x 0.53 mm i.d. 0.5 μm). The column temperature was programmed from 150 °C after an initial hold of 4 minutes to 200 °C at a rate of 4 °C/ minute, from 200 °C to 280 °C at a rate of 20 °C/ minute

and maintained at 280 °C for 10 minute. The injector and detector were maintained at 250 °C and 325 °C, respectively. Nitrogen was used as carrier gas at a pressure of 6 psi. Quantification of OCPs (Aldrin, α-BHC, β-BHC, γ-BHC, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE *o,p'*-DDT, *p,p'*-DDT, dieldrin, endrin, heptachlor, heptachlor epoxide) was made through the area of sample peaks that matched with OCP standards. Blank runs were made to ensure purity of the system. The recovery of OCP ranged from 80 to 102% and the results have not been corrected for percentage recovery.

4.3.2.5. Organochlorine pesticides in mussel tissue

Organochlorine pesticides from the samples were extracted by a standard method (FDA, 1999). Tissue samples collected from 30 mussels (size >50 mm) from Someshwara and Surathkal mussel beds were pooled and analysed separately.

Briefly, fat from homogenised edible portions (50 g) was extracted with petroleum ether (60-80 °C). The petroleum ether extract was concentrated to 10 ml and an aliquot containing less than 3 g fat was taken for further clean up. The OCPs in the fat were partitioned between petroleum ether (60-80 °C) and acetonitrile (HPLC grade) followed by fractionation on florisil column. After washing the column with petroleum ether the organochlorines were eluted with petroleum ether containing 6% and 15% diethyl ether. The extracts were pooled, evaporated and made up to 1 ml in petroleum ether.

Quantification of OCPs was made on gas chromatograph (Perkin Elmer-Autosystem XL) equipped with ^{63}Ni electron capture detector (ECD) and Elite-5 column (Perkin Elmer, 30 m x 0.53 mm i.d. 0.5 μm) as detailed above for the seawater. The pesticide concentrations in tissue were expressed as ppb on a wet weight basis.

4.3.3. Neutral Red Retention Assay (NRRA)

Neutral red is a cytotoxic weak base (Lowe and Pipe, 1994) and is used in the neutral red retention assay (Lowe *et al.*, 1992) as a stressor to test the structural integrity of the lysosomal membranes. The neutral red dye (3-amino-7-dimethylamino-2-methylphenazine hydrochoride) has been found in extensive use in *in vitro* toxicity testing for a number of years (Borenfreund and Puerner, 1985). The dye retention time has been used as a comparative measure of lipid membrane damage (Lowe *et al.*, 1995b). The assay was performed in mussel samples collected during February-March, June-July and September-October. Mussels were cleared off epibionts immediately after the transfer of samples to the laboratory.

The lysosomal neutral red assay was performed as described by Lowe *et al.* (1995a). The mussel valves were prised apart with a fixed blade scalpel and 0.5 ml of haemolymph was withdrawn from the posterior adductor muscle with a 1.0

ml hypodermic syringe, containing 0.5 ml of physiological saline. The needle was discarded and the contents were transferred to a 2 ml siliconized Eppendorf tube and mixed thoroughly. 50 µl aliquot of the cell suspension was dispensed onto a poly-L-Lysine coated glass slide and placed on a rack in a humidity chamber for 15 minutes to allow the cells to attach to the slide. A stock solution of neutral red was freshly prepared by dissolving 20 mg of the dye in 1 ml of DMSO (dimethylsulfoxide). A working solution was then prepared by diluting 10 µl of the stock solution with 5 ml of the physiological saline. After 15 minutes the slides were removed from the humidity chamber, excess of solution was carefully tipped off and 40 µl of the neutral red working solution was added to the area containing the attached cells. A cover slip was applied after 15 minutes incubation in a light proof chamber. The granular haemocytes were observed under light microscope at 15 minutes intervals to determine the loss of dye from the lysosomes into the cytosol. The test was concluded when 50% or more of the cells exhibited neutral red dye spread into the whole cytosol. The retention time of the neutral red by the lysosomes was recorded by estimating the proportion of cells displaying leakage from the lysosomes into the cytosol (Lowe and Pipe, 1994). A random sample of 18-24 mussels collected from Someshwara and Surathkal mussel beds was assayed blind for lysosomal membrane stability. All assays were conducted within 48 h of sampling from the mussel beds.

4.3.4. Bioaccumulation factor

Bioaccumulation factor or bioconcentration factor (BF), which is the number of times the mussel accumulate contaminants in comparison with the levels in the ambient seawater was calculated for trace metals by the formula (Nair, 1984):

$$\text{BF-Tissue/water} = \frac{\text{Concentration of metal in the tissue}}{\text{Concentration of metal in the seawater}}$$

$$\text{BF-Shell/water} = \frac{\text{Concentration of metal in the shell}}{\text{Concentration of metal in the seawater}}$$

$$\text{BF-Shell/tissue} = \frac{\text{Concentration of metal in the shell}}{\text{Concentration of metal in the tissue}}$$

4.3.5. Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the individual contaminant concentration and lysosomal retention time among the mussel beds. Pearson's correlation analysis was performed to find out the relationship between trace metals in ambient water with that in mussel tissue. SPSS (13.0) software was used for all the analysis.

4.4. Results

4.4.1. Trace metals

4.4.1.1. Trace metals in seawater

Mean trace metal concentrations of Cd, Pb, Cu, Zn, Ni and Fe in seawater (ppm) from Someshwara and Surathkal mussel beds are presented in Table 4.1. The concentrations of trace metals considered were higher in mussel tissue and shells than in the seawater collected from the mussel beds.

The mean concentration of Cd in seawater samples from Someshwara ranged from nd (below detectable limits) to 0.0027, Pb from 0.00014 to 0.0046, Cu from 0.00031 to 0.0064, Zn from 0.0009 to 0.0333, Ni from 0.0008 to 0.0114 and Fe from 0.04 to 5.87 ppm. In the seawater from Surathkal mussel beds, the concentrations of Cd ranged from 0.00007 to 0.0066, Pb from 0.0006 to 0.0105, Cu ranged from 0.0012 to 0.012, Zn from 0.0039 to 0.0299, Ni from 0.0009 to 0.0055 and Fe from 0.0391 to 2.3953 ppm. Cd showed relatively very low concentrations in the seawater of the mussel beds and the distribution pattern being in the order of Fe>Zn>Cu>Ni>Pb>Cd at Someshwara and Fe>Zn>Cu>Pb>Ni>Cd at Surathkal.

Analysis of variance of trace metal concentrations in seawater between the two mussel beds is presented in Table 4.2. Cd concentrations in seawater were 2.8 times higher in Surathkal (0.0005±0.0007 ppm) than the levels in Someshwara (0.0002±0.0003 ppm) mussel bed. Surathkal seawater (0.0019±0.0013 ppm) had significantly higher (1.5 times) Pb concentrations ($p<0.05$) than in Someshwara (0.0014±0.0010 ppm). Similarly the Cu and Zn concentrations in seawater of the Surathkal mussel beds were 1.2 and 1.7 times higher than that of Someshwara. Between the mussel beds, Ni and Fe levels in seawater presented the least spatial trends during the study.

4.4.1.2. Trace metals in mussel tissue

The trace metal concentrations in the soft tissues of *P. viridis* are shown in Table 4.3. The pattern of distribution of trace metals in the mussel tissue was Fe>Zn>Cu>Pb>Ni>Cd at Someshwara and at Surathkal it was in the order of Fe>Zn>Cu>Ni>Cd>Pb. The soft tissue concentration of Cd in green mussels from Someshwara varied from nd to 0.57, Pb from nd to 6.72, Cu from nd to 7.06, Zn from 1.99 to 34.7, Ni from nd to 0.9 and Fe from nd to 223.8 ppm wet wt. Similarly the soft tissue concentrations of Cd in mussels from Surathkal varied from nd to 3.35, Pb from nd to 3.68, Cu from nd to 12.37, Zn from 3.79 to 43.48, Ni from nd to 12.23 and Fe from nd to 46.62 ppm wet wt.

The analysis of variance of the mean concentrations of trace metals in the mussel tissue of *P. viridis* from Someshwara and Surathkal mussel beds is presented in Table 4.4. The concentration of Cd in mussel tissue (3.1 times) was higher at Surathkal but the differences were not significant. Fe, Ni, Cd and Zn levels of mussel tissue samples originating from Surathkal were higher compared to Someshwara while Cu and Pb levels were lower. Statistical analysis of concentration of metals revealed no significant difference in the levels of metal between the two mussel beds.

4.4.1.3. Trace metals in mussel shell

Cd, Pb and Ni were the least abundant metals in mussel tissue as well as shells (Table 4.3). At Someshwara Cd content in the mussel shells were observed in the range of nd to 3.87, Pb from nd to 20.95, Cu from nd to 13.75, Zn from nd to 37.72, Ni from nd to 1.93 and Fe from nd to 73.92 ppm wet wt. In the mussel shell samples from Surathkal the Cd ranged from nd to 6.98, Pb from nd to 19.16, Cu from nd to 19.43, Zn from nd to 54.2 and Fe from nd to 30.96 ppm wet wt. The distribution pattern of trace metals at Someshwara was in the order of Fe>Zn>Cu>Cd>Pb>Ni where as at Surathkal it was in the order of Zn>Cu>Fe>Cd>Pb>Ni.

Cd, Cu and Pb concentrations were higher in mussel shell than in the soft tissues; conversely Fe and Zn concentrations were noticeably higher in the soft tissues than in the shell. The results of the analysis of variance of the mean concentrations of Cd, Cu, Fe, Ni, Pb and Zn in the mussel shell from the mussel beds are presented in Table 4.4.

The concentration of Cd in mussel shell (1.4 times) was higher at Surathkal than at Someshwara whereas, the concentration of all the other metals studied was found to be low at Surathkal. Analysis of variance indicated significant difference in the levels of Cd, Cu and Fe between the two mussel beds.

4.4.1.4. Bioaccumulation factor

Bioaccumulation factor of trace metals in mussel tissue (BF-tissue/water) and shell (BF-shell/water) from Someshwara and Surathkal mussel beds are presented in Table 4.5. The bioaccumulation factor varied with different trace metals and it presented variation between the shell and soft tissue. Among the trace metals, the highest bioaccumulation in tissue as well as shell was observed for Cu, Cd and Zn at Someshwara and Surathkal mussel beds. In mussel tissue Cd showed a BF 10^2 times and Cu showed a BF 10^3 times as compared to the surrounding waters. Mussel shells showed highest ratios for Cd and Cu, accumulating 10^3 times more than the surrounding waters. Comparatively low BF was observed for Pb in tissue than in shells.

Table 4.1. Mean trace metal concentrations in seawater of the mussel beds off Someshwara and Surathkal.

Trace Metals	Seawater (ppm)			
	Someshwara		Surathkal	
	Mean ± SD	Range	Mean ± SD	Range
Cd	0.0002±0.0003	0.0-0.0027	0.0005±0.0007	0.00007-0.0066
Pb	0.0014±0.0010	0.00014-0.0046	0.0019±0.0013	0.0006-0.0105
Cu	0.0023±0.0016	0.00031-0.0064	0.0027±0.0017	0.0012-0.0120
Zn	0.0090±0.0068	0.0009-0.0333	0.0149±0.0054	0.0039-0.0299
Ni	0.0019±0.0015	0.0008-0.0114	0.0019±0.0009	0.0009-0.0055
Fe	0.6536±0.9078	0.04-5.8700	0.4917±0.4414	0.0391-2.3953

Table 4.2. Analysis of variance of trace metal concentrations of seawater of the mussel beds (station) off Someshwara and Surathkal.

Source		**Sum of squares**	**Degrees of freedom**	**Mean sum of squares**	**F value computed**	***p***
Cd x station	Combined	3.49E-06	1	3.49E-06	**10.86**	*0.001*
	Within Groups	4.76E-05	148	3.22E-07		
	Total	5.11E-05	149			
Pb x station	Combined	1.03E-05	1	1.03E-05	**7.345**	*0.008*
	Within Groups	2.07E-04	148	1.40E-06		
	Total	2.17E-04	149			
Cu x station	Combined	8.93E-06	1	8.93E-06	3.366	*0.069*
	Within Groups	3.93E-04	148	2.65E-06		
	Total	4.02E-04	149			
Zn x station	Combined	1.28E-03	1	1.28E-03	**33.84**	*0.000*
	Within Groups	5.60E-03	148	3.79E-05		
	Total	6.89E-03	149			
Ni x station	Combined	8.03E-08	1	8.03E-08	0.053	*0.818*
	Within Groups	2.25E-04	148	1.52E-06		
	Total	2.25E-04	149			
Fe x station	Combined	9.81E-01	1	9.81E-01	1.878	*0.173*
	Within Groups	7.73E+01	148	5.22E-01		
	Total	7.83E+01	149			

Table 4.3. Mean trace metal concentrations of soft tissue and shell of *P. viridis* of the mussel beds off Someshwara and Surathkal.

Trace Metals	Mussel tissue (ppm)			
	Someshwara		Surathkal	
	Mean ± SD	Range	Mean ± SD	Range
Cd	0.03±0.10	nd-0.57	0.10±0.46	nd-3.35
Pb	0.11±0.84	nd-6.72	0.06±0.48	nd-3.68
Cu	3.38±1.97	nd-7.06	2.98±3.07	nd-12.37
Zn	14.07±5.28	1.9-34.7	14.94±7.21	3.79-43.48
Ni	0.041±0.17	nd-0.90	0.26±1.64	nd-12.23
Fe	30.56±42.4	nd-223	33.6±46.6	nd-172.73

Trace Metals	Mussel shell (ppm)			
	Someshwara		Surathkal	
	Mean ± SD	Range	Mean ± SD	Range
Cd	1.18±1.27	nd-3.87	1.68±1.71	nd-6.98
Pb	1.08±3.78	nd-20.95	0.91±3.45	nd-19.16
Cu	7.22±3.36	nd-13.75	5.66±5.33	nd-19.43
Zn	7.70±8.70	nd-37.72	6.11±9.12	nd-54.2
Ni	0.08±0.35	nd-1.93	nd	nd
Fe	9.85±17.22	nd-73.92	4.01±6.79	nd-30.96

nd - below detectable limits

Table 4.4. Analysis of variance of trace metal concentrations in soft tissue and shell of *P. viridis* of the mussel beds (station) off Someshwara and Surathkal.

Source Mussel tissue		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Cd x station	Combined	0.1	1	0.15	1.40	*0.239*
	Within Groups	12.8	120	0.11		
	Total	13.0	121			
Pb x station	Combined	0.1	1	0.05	0.11	*0.742*
	Within Groups	58	120	0.48		
	Total	58	121			
Cu x station	Combined	4.9	1	4.93	0.75	*0.387*
	Within Groups	783	120	6.53		
	Total	788	121			
Zn x station	Combined	23	1	22.9	0.58	*0.447*
	Within Groups	4718	120	39.3		
	Total	4741	121			
Ni x station	Combined	1.4	1	1.43	1.11	*0.295*
	Within Groups	155	120	1.29		
	Total	156	121			
Fe x station	Combined	289	1	289	0.15	*0.703*
	Within Groups	237360	120	1978		
	Total	237648	121			

Source Mussel shell		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Cd x station	Combined	8.8	1	8.8	**3.94**	*0.049*
	Within Groups	305	136	2.2		
	Total	314	137			
Pb x station	Combined	1.0	1	1.0	0.08	*0.783*
	Within Groups	1785	136	13		
	Total	1786	137			
Cu x station	Combined	84	1	84	**4.32**	*0.04*
	Within Groups	2647	136	19		
	Total	2731	137			
Zn x station	Combined	87	1	87	1.10	*0.297*
	Within Groups	10782	136	79		
	Total	10868	137			
Ni x station	Combined	0.2	1	0.2	3.43	*0.066*
	Within Groups	8.8	136	0.1		
	Total	9.0	137			
Fe x station	Combined	1177	1	1177	**6.66**	*0.011*
	Within Groups	24046	136	177		
	Total	25223	137			

Table 4.5. Bioaccumulation factor (BF) of trace metals in *P. viridis* from mussel beds off Someshwara and Surathkal.

	Mussel bed	Cd	Pb	Cu	Zn	Ni	Fe
BF-Tissue/water	Someshwara	200	73	1495	1555	21	47
	Surathkal	219	32	1083	1003	136	68
BF-Shell/water	Someshwara	7202	749	3196	852	41	15
	Surathkal	3590	463	2059	411	0	8
BF -Shell/tissue	Someshwara	36.02	10.28	2.14	0.55	1.95	0.32
	Surathkal	16.38	14.33	1.90	0.41		0.12

Table 4.6. Correlation coefficient (Pearson's) between concentrations of trace metals in seawater, mussel tissue and shells of *P. viridis* from the mussel bed off Someshwara and Surathkal

	Shell-Cd	Tissue-Cd	Water-Cd	Shell-Cu	Tissue-Cu	Water-Cu	Shell-Fe	Tissue-Fe	Water-Fe	Shell-Ni	Tissue-Ni	Water-Ni	Shell-Pb	Tissue-Pb	Water-Pb	Shell-Zn	Tissue-Zn	Water-Zn	Shell-Mn	Tissue-Mn	Water-Co
Shell-Cd	1.000	.459(**)	.408(*)	.769(**)	.645(**)	.645(**)	.689(**)	.369(*)	0.324	.653(**)	.422(*)	.386(*)	0.319	0.124	.524(**)	.758(**)	.793(**)	.489(**)	.519(**)	.481(**)	0.310
Tissue-Cd		1.000	0.023	.372(*)	.493(**)	.746(**)	0.295	.372(*)	0.123	0.115	0.095	0.143	-0.071	-0.088	.787(**)	0.236	0.228	0.239	0.062	0.074	0.048
Water-Cd			1.000	0.003	-0.052	0.327	0.220	.437(*)	0.299	0.308	0.196	0.288	.696(**)	.454(**)	.389(*)	0.134	.373(*)	.502(**)	0.051	0.093	.330(*)
Shell-Cu				1.000	.871(**)	.415(*)	.506(**)	0.037	0.167	.533(**)	0.310	0.190	-0.104	-0.169	0.236	.622(**)	.625(**)	0.138	.680(**)	.641(**)	0.128
Tissue-Cu					1.000	.387(*)	0.331	0.145	0.131	0.281	0.163	0.104	-0.182	-0.189	0.282	.439(*)	.435(*)	0.070	.685(**)	.648(**)	0.058
Water-Cu						1.000	.545(**)	.512(**)	.478(**)	0.211	0.303	.517(**)	0.158	0.119	.926(**)	.475(**)	.618(**)	.716(**)	0.103	0.066	.438(**)
Shell-Fe							1.000	.393(*)	0.307	0.296	0.164	.345(*)	.365(*)	.375(*)	.463(**)	.715(**)	.538(**)	.611(**)	0.200	0.144	0.262
Tissue-Fe								1.000	.539(**)	-0.094	-0.013	.419(*)	.643(**)	.660(**)	.643(**)	0.250	0.226	.542(**)	0.019	0.013	.464(**)
Water-Fe									1.000	0.018	0.008	.927(**)	0.133	0.128	.413(*)	0.274	.427(*)	.655(**)	0.138	0.106	.962(**)
Shell-Ni										1.000	0.335	0.240	0.264	-0.070	0.071	.549(**)	.588(**)	0.153	.370(*)	.446(**)	0.131
Tissue-Ni											1.000	-0.030	0.081	0.045	0.287	.618(**)	.646(**)	0.106	.430(*)	0.336	-0.001
Water-Ni												1.000	0.155	0.080	.397(*)	0.331	.471(**)	.711(**)	0.066	0.067	.956(**)
Shell-Pb													1.000	.871(**)	0.311	0.306	0.200	.456(**)	-0.052	-0.008	0.142
Tissue-Pb														1.000	0.307	0.297	0.049	.415(*)	-0.103	-0.109	0.076
Water-Pb															1.000	.408(*)	.475(**)	.688(**)	0.040	0.019	0.322
Shell-Zn																1.000	.775(**)	.500(**)	.550(**)	.452(**)	0.230
Tissue-Zn																	1.000	.605(**)	.580(**)	.517(**)	.410(*)
Water-Zn																		1.000	0.045	-0.006	.612(**)
Shell-Mn																			1.000	.957(**)	0.069
Tissue-Mn																				1.000	0.060
Water-Co																					1.000

4.4.1.5. Influence of trace metal levels in ambient waters on the mussel tissue and shell concentrations

Correlation between concentrations of trace metals in seawater, mussel tissue and shell was examined to study their interrelationships (Table 4.6). Significant positive correlations were observed between metal levels in seawater and in mussel tissue. Cd, Cu and Zn content in seawater exhibited significant positive correlation ($p<0.05$) with that in mussel shell. Significant positive correlation was observed between Cu, Fe and Zn in seawater ($p<0.05$) and levels of these metals in mussel tissue. These results indicate that the trace metal concentrations in the habitat waters have a direct bearing on their levels in the mussels.

4.4.2. Organochlorine pesticides

4.4.2.1. Organochlorine pesticides in seawater

Concentrations of organochlorine pesticides (OCPs) in seawater and mussel samples collected from Someshwara and Surathkal mussel beds are presented in Table 4.7. In general, many of the OCPs were below the detection limits in seawater and mussel samples from the study area. The mean seawater concentration of ∑OCPs at Someshwara was 0.164±0.236 ppb and at Surathkal it was 0.081±0.088 ppb. Endrin was the major OCP detected in seawater both at Someshwara and Surathkal mussel beds, where it contributed 40% (0.0657±0.12 ppb) and 64% (0.0517±0.084 ppb) to the OCP concentration respectively. DDT and its metabolites (∑DDT) contributed 40% to the OCPs of Someshwara, whereas at Surathkal it formed only 8.8%. The mean concentrations were 0.0671±0.109 ppb at Someshwara and 0.0069±0.010 ppb at Surathkal. At Someshwara, *p,p'*-DDT contributed only 3.8% (0.0063±0.014 ppb), whereas, *p,p'*-DDE and *o,p'*-DDD contributed 17.8% (0.0292±0.043 ppb) and 19.35% (0.0317±0.053 ppb) to the OCPs concentrations. DDT and DDD were not detected at Surathkal and only *p,p'*-DDE (0.0069±0.010 ppb) represented the ∑DDT fraction. α-BHC contributed the major share to ∑BHC fraction in the seawater of mussel beds forming 13% and 27% of the total OCPs at Someshwara and Surathkal respectively. Detectable concentrations of γ-BHC were measured in seawater, where it formed only >0.1% (>0.0001 ppb) of the OCP concentrations at Someshwara and Surathkal. β-BHC was below the detectable limits in seawater samples from the mussel beds. At Someshwara, heptachlor concentration was 0.0013±0.003 ppb (0.8%) and heptachlor epoxide was 0.005±0.006 ppb (3.1%), whereas, at Surathkal heptachlor and heptachlor epoxide were below the detectable limits in seawater samples. Aldrin contributed 2% to the OCP concentrations at Someshwara, at 0.0033±0.007 ppb levels whereas; it was below the detectable limits in the water samples from Surathkal mussel beds. Dieldrin was not detected in water samples from both the mussel beds. Analysis of variance showed no significant difference in the OCPs concentrations of seawater between the mussel beds (Table 4.8).

4.4.2.2. Organochlorine pesticide in mussel tissue

The mean tissue concentration of ∑OCPs at Someshwara was 0.431±0.019 ppb wet wt. Similarly, at Surathkal the OCP concentration was 0.536±0.023 ppb wet wt. In mussel tissue, heptachlor epoxide contributed the maximum share to the ∑OCPs, forming 98.2% (0.4237±0.018 ppb wet wt) and 60.8% (0.3260±0.014 ppb wet wt) respectively at Someshwara and Surathkal. Among BHC, only α-BHC was detected in mussel tissue forming 1.8% (0.0076±0.001 ppb wet wt) of the OCP concentration at Someshwara. At Surathkal, α-BHC concentration, was relatively higher (0.1080±0.153 ppb wet wt) when, compared to Someshwara and it contributed 27% to the OCP concentrations in the mussel tissue. Aldrin contributed 19% to the OCP concentrations at Surathkal (0.102±0.144 ppb wet wt) whereas, it was below the detectable limits in the tissue samples from Someshwara. DDT and its derivatives were not detected in mussel tissue samples from Someshwara and Surathkal mussel beds during the study. Similarly, β-BHC, Endrin and Dieldrin were below the detectable limits in the tissue samples from both the mussel beds. Analysis of variance in tissue concentrations of OCPs between the mussel beds showed no significant difference except in Heptachlor epoxide levels (Table 4.9).

4.4.3. Lysosomal membrane stability

The results of the neutral red retention assay of haemocytes isolated from the mussels are presented in Fig. 4.1. Variations in the magnitude of the biomarker response did not remain statistically significant between the three batches of samples from the same mussel bed analysed during the study. Therefore, the lysosomal membranes destabilization time for Someshwara and Surathkal were estimated from the pooled data. The lysosomes from the green mussel population in Someshwara had the capacity to retain the dye for an average retention time of 122±11 minutes. Similarly mussels from the Surathkal gave an average retention time of 127±8 minutes. Analysis of variance in the retention time revealed no significant differences between the mussel beds (Table 4.10).

Table 4.7. Concentration of organochlorine pesticides in seawater and mussel tissue of *P. viridis* from the mussel bed off Someshwara and Surathkal.

Organo-chlorine pesticides	Seawater (ppb)				Mussel tissue (ppb wet wt.)			
	Someshwara		Surathkal		Someshwara		Surathkal	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
α-BHC	0.0214	0.016	0.0219	0.012	0.0076	0.001	0.1080	0.153
β-BHC	nd	nd	nd	nd	nd	nd	nd	nd
γ-BHC	0.0001	0.000	0.0001	0.000	nd	nd	nd	nd
Heptachlor	0.0013	0.003	nd	nd	nd	nd	nd	nd
Aldrin	0.0033	0.007	nd	nd	nd	nd	0.1020	0.144
Heptachlor epoxide	0.0050	0.006	nd	nd	0.4237	0.018	0.3260	0.014
Dieldrin	nd	nd	nd	nd	nd	nd	nd	nd
Endrin	0.0657	0.120	0.0517	0.084	nd	nd	nd	nd
o,p'-DDT	nd	nd	nd	nd	nd	nd	nd	nd
p,p'-DDT	0.0063	0.014	nd	nd	nd	nd	nd	nd
p,p'-DDE	0.0292	0.043	0.0069	0.010	nd	nd	nd	nd
o,p'-DDD	0.0317	0.053	nd	nd	nd	nd	nd	nd
p,p'-DDD	nd	nd	nd	nd	nd	nd	nd	nd
∑BHC	0.0215	0.016	0.0221	0.012	0.0076	0.001	0.1080	0.153
∑DDT	0.0671	0.109	0.0069	0.010	nd	nd	nd	nd
DDE/DDT	0.4348	nd	1.0000	nd	nd	nd	nd	nd
∑OCP	0.1639	0.243	0.0808	0.088	0.4313	0.019	0.5360	0.023

nd-below detectable limits

Table 4.8. Analysis of variance of concentrations of organochlorine pesticides in seawater of the mussel beds (station) off Someshwara and Surathkal.

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
α-BHC x station	Combined	7E-07	1	7E-07	0.00	*0.956*
	Within Groups	0.002	8	0.000		
	Total	0.002	9			
γ-BHC x station	Combined	0	1	0	0.00	*1.000*
	Within Groups	6E-07	8	7E-08		
	Total	6E-07	9			
Heptachlor x station	Combined	4E-06	1	4E-06	1.00	*0.347*
	Within Groups	4E-05	8	4E-06		
	Total	4E-05	9			
Aldrin x station	Combined	3E-05	1	3E-05	1.00	*0.347*
	Within Groups	0.000	8	3E-05		
	Total	0.000	9			
Heptachlor epoxide x station	Combined	6E-05	1	6E-05	4.02	*0.080*
	Within Groups	0.000	8	2E-05		
	Total	0.000	9			
Endrin x station	Combined	0.000	1	0.000	0.05	*0.837*
	Within Groups	0.086	8	0.011		
	Total	0.087	9			
p,p'-DDT x station	Combined	1E-04	1	1E-04	1.00	*0.347*
	Within Groups	0.001	8	1E-04		
	Total	0.001	9			
p,p'-DDE x station	Combined	0.001	1	0.001	1.26	*0.294*
	Within Groups	0.008	8	0.001		
	Total	0.009	9			
o,p'-DDD x station	Combined	0.003	1	0.003	1.78	*0.219*
	Within Groups	0.011	8	0.001		
	Total	0.014	9			
∑BHC x station	Combined	7E-07	1	7E-07	0.00	*0.956*
	Within Groups	0.002	8	0.000		
	Total	0.002	9			
∑DDT x station	Combined	0.009	1	0.009	1.52	*0.253*
	Within Groups	0.048	8	0.006		
	Total	0.057	9			
DDE/DDT x station	Combined	0.107	1	0.107	0.57	*0.471*
	Within Groups	1.498	8	0.187		
	Total	1.605	9			
∑OCP x station	Combined	0.017	1	0.017	0.52	*0.492*
	Within Groups	0.267	8	0.033		
	Total	0.284	9			

Table 4.9. Analysis of variance of concentrations of organochlorine pesticides in mussel tissue between Someshwara and Surathkal mussel beds (station).

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
α-BHC x station	Combined	0.010	1	0.010	0.86	0.451
	Within Groups	0.023	2	0.012		
	Total	0.033	3			
Aldrin x station	Combined	0.010	1	0.010	1.00	0.423
	Within Groups	0.021	2	0.010		
	Total	0.031	3			
Heptachlor epoxide x station	Combined	0.010	1	0.010	**37.26**	0.026
	Within Groups	0.001	2	0.000		
	Total	0.010	3			
∑BHC x station	Combined	0.010	1	0.010	0.86	0.451
	Within Groups	0.023	2	0.012		
	Total	0.033	3			
∑OCP x station	Combined	0.006	1	0.006	15.15	0.060
	Within Groups	0.001	2	0.000		
	Total	0.006	3			

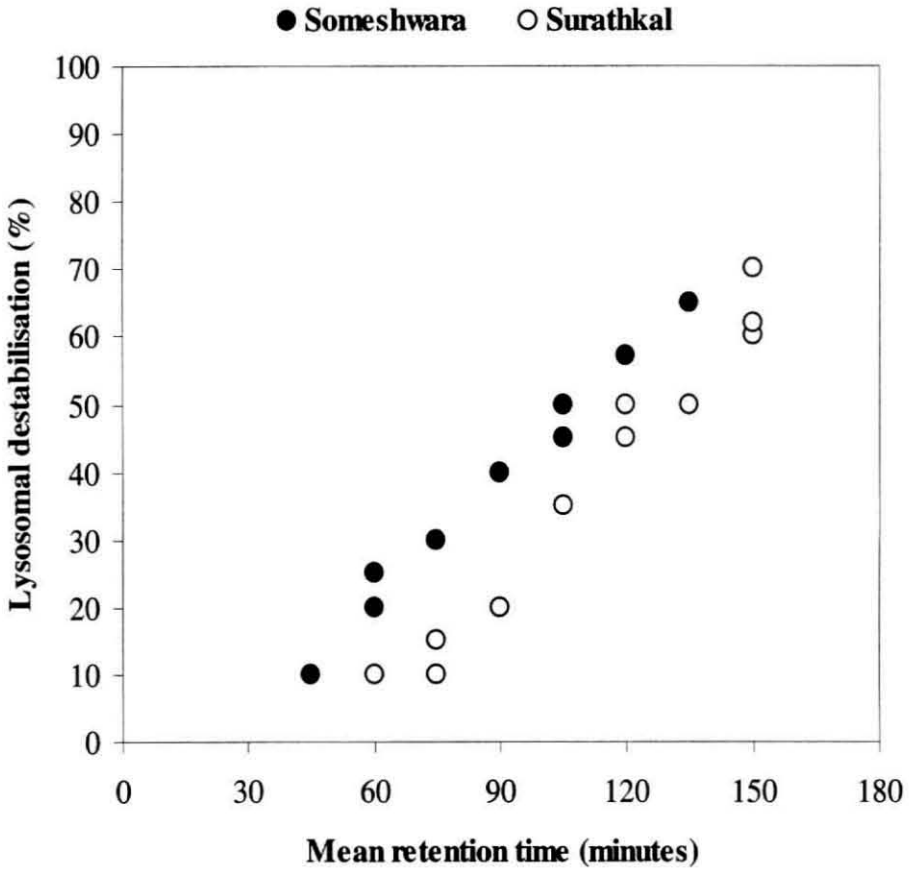

Fig. 4.1. Lysosomal membrane stability expressed as neutral red retention time in green mussel haemocytes.

Table 4.10. Analysis of variance of neutral red retention time in mussel haemocytes of *P. viridis* between mussel beds (station) off Someshwara and Surathkal

Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Time x station	Combined	75	1	75	0.769	*0.401*
	Within Groups	975	10	98		
	Total	1050	11			

4.5. Discussion

4.5.1. Trace metals

The evidence of trace metal accumulations and their distribution in the soft tissue of green mussel is documented from the coastal waters of Karnataka by Krishnakumar *et al.* (1990a,b), Krishnakumar and Pillai (1990), Krishnakumar *et al.* (1998), Krishnakumar *et al.* (2004b) and Sasikumar *et al.* (2006).

Along the Dakshina Kannada coast, no spatial variation was observed between the mussel beds of Someshwara and Surathkal in the trace metal concentration in mussel tissue. In seawater samples from Someshwara and Surathkal significant difference was observed only in Cd, Pb and Zn levels.

Several studies have supported the idea that shells can record the metal concentration history of the environment where, the shelled organisms live, assuming that the kinetics of metal loss is considerably slower in the calcium carbonate structure than in the soft tissue (Lares *et al.*, 2005). The variability in trace metal concentrations in the shells of *P. viridis* from the two mussel beds was observed in Cd, Cu and Fe. Although the shell accumulated elevated concentrations of trace metals, the ratios of Zn and Fe in shell to soft tissues were low in the mussel beds indicating dissimilar rates in their bioaccumulation.

In mussel tissue, the trace metal distribution based on concentration was Fe>Zn>Cu>Pb>Ni>Cd at Someshwara and Fe>Zn>Cu>Cd>Ni>Pb at Surathkal. In mussel shell the distribution was Fe>Zn>Cu>Cd>Pb>Ni at Someshwara and Zn>Cu>Fe>Cd>Pb>Ni at Surathkal. Interest in metals like Zn, Cu and Fe which, are required for metabolic activity in organisms lies in the narrow "window" between their essentiality and toxicity. Other trace metals like Cd and Pb may exhibit extreme toxicity even at low levels under certain conditions, thus, necessitating regular monitoring of sensitive aquatic environments (Peerzada *et al.*, 1990).

High Fe concentrations were evident in both the mussel beds and Fe accumulation was the highest among the metals studied. Being an essential metal abundant in any environment, the presence of Fe in natural waters can be attributed to the weathering of rocks and minerals. High levels of Fe are known to occur in sediments and a proportion of Fe in sediment may be due to natural erosion (Boggs, 1987). The bioaccumulation indices of Fe in mussel tissue and shells were relatively lower compared to the other trace metals due to its higher concentrations in seawater as well as in the mussels. The bioaccumulation indices of Fe in the tissue were higher than that of the shell from both the mussel beds and a stronger correlation was observed between the concentration of Fe in seawater and that of the mussel tissue. The Fe levels in the seawater and mussel tissue samples from the Someshwara and Surathkal mussel beds were high and

were of similar magnitude as reported from the region by Sasikumar *et al.* (2006) as well as from the earlier studies on green mussels from the west coast of India (Table 4.12). However, when permissible levels of Fe are concerned no guidelines are available regarding its limit in shellfish.

Zinc levels in seawater were high in both the mussel beds with significantly higher levels at Surathkal, but no significant difference in Zn concentrations in the soft tissue as well as mussel shells were noticed between the mussel beds. Generally the effluent discharges from metallurgic industry, electroplating industry, petrochemical industry, run offs from agricultural areas, dredging of harbour and municipal wastes are the sources of Zinc in coastal waters. Many Zinc compounds are soluble in seawater and hence are accumulated in marine organisms. Relatively consistent tissue concentrations of Zn were observed in mussels in earlier studies with least spatial difference in its distribution through out the Karnataka coast (Sasikumar *et al.,* 2006). Similar to Fe, Zn concentrations in the mussel were high and comparable with earlier studies (Table 4.12) reported by Krishnakumar *et al.* (1998) and relatively lower than those reported by other authors from Indian waters (Zingde *et al.,* 1976; Rajendran *et al.,* 1988; Senthilnathan *et al.,* 1998; Radhakrishnan, 1994 and Tewari *et al.,* 2000, 2001).

Among the trace metals studied, bioaccumulation factor for Zn in tissue was the highest in both the beds. Though there was a relatively higher seawater concentration of Zn at Surathkal, the bioaccumulation factor of Zn in mussel tissue and shell was lower than that of Someshwara. The high values for Zn were also found in the seawater (Krishnakumar *et al.*, 2004a) and sediments along coastal waters of Karnataka (Krishnakumar *et al.,* 1998). However, mussels are known to regulate zinc uptake and are therefore reported as not reliable indicator for zinc contamination (Lobel *et al.*, 1982 and Julshamn, 1981). Even then, significant positive correlations between Zn in seawater and tissue concentrations imply that Zn is accumulated in soft tissue and shell in green mussels in a proportion similar to its availability in ambient waters. Although Zn uptake is regulated by the soft tissue, it was observed that the incorporated Zn remained in the soft tissue, suggesting the use of *P. viridis* as a potential biomonitoring agent for assessing Zn contamination over extended periods. Zn in aquatic environment predominantly binds to suspended material before settling in sediments. Szefer *et al.* (2004) observed a positive correlation between Zn in blue mussel tissue and their levels in suspended matter and attributed it to the Zn levels in suspended particulate matter, which constitute the potential food for the mussels as filter feeders.

The mean Zn concentrations observed in the seawater of Someshwara (0.009 ppm) and Surathkal (0.0149 ppm) mussel beds were much lower than the the LC_{50} value of 3.20 ppm for *P. viridis* reported by Yap *et al.* (2004b). Regarding the level of Zn in mussel tissue the observed levels were well below the limit set

by WHO and EU and the concentrations in seawater were below the standards set by EC (Table 4.11).

Concentration of Cu in the mussel tissue and shell presented significant positive correlations with its level in seawater. Although, no statistically significant difference in Cu concentration of mussel tissue was observed between the mussel beds, it was relatively higher at Someshwara. Cu concentrations in mussel samples from the study area indicated marginal increase when compared with previous studies (Krishnakumar *et al.,* 1998 and Sasikumar *et al.,* 2006) from non-urban sites of Karnataka. On the other hand, the Cu levels in green mussel were lower when compared to industrial and urban sites of Karnataka (Krishnakumar *et al.,* 1998) and those reported in other studies along the coastline (Zingde *et al.,* 1976; Rajendran *et al.,* 1988; Senthilnathan *et al.,* 1998; Radhakrishnan, 1994 and Tewari *et al.,* 2000, 2001).

The bioaccumulation of Cu in mussel tissue and shell was relatively higher in both the beds but higher accumulation was noticed in shells than tissue of mussels from both the beds. A strong positive correlation was observed between the Cu concentration in seawater, mussel tissue and shells. The Cu concentrations in the mussel beds can be related to inflows from non-point sources around the mussel beds. Copper is the most common metal found at toxic concentrations in several harbours (NCDEM, 1990, 1991). It is used as biocides in antifoulant paints either as copper, copper oxide or copper sulphate. There is heavy boat traffic in the harbour areas near the mussel beds and the use of copper-based antifoulants in these crafts may become a point of concern due to its potential for accumulation in sediments and its toxicity to benthic organisms. Similar to Zn, a positive correlation was observed between Cu in blue mussels and their levels in suspended matter by Szefer *et al.* (2004) which was attributed to the suspended particulate matter.

The LC_{50} value of Cu reported for *P. viridis* is 0.25 ppm (Yap *et al.*, 2004b). The mean concentration of 0.0023 and 0.0003 ppm of Cu observed in the coastal waters of Someshwara and Surathkal was nearly 10^2 times lower than LC_{50} value. From the food safety point of view, the tissue levels of Cu in Someshwara and Surathkal mussel beds were well below the WHO standards and can be considered as safe for human consumption. No guidelines for Cu content are given by US FDA.

At Someshwara mussel beds, Cd levels in seawater and mussel tissue were lower than at Surathkal. Significant positive correlations of Cd in seawater and mussel indicate that the increases in Cd concentrations in mussel tissue and shells at Surathkal corresponded to the higher Cd availability in the seawater. Relatively higher level of Cd in Surathkal mussel bed indicates a higher input of Cd in to the coastal waters. Though the Cd concentrations were very low in seawater, bioaccumulation of Cd was the highest in the mussel shells in both Someshwara and Surathkal beds. Compared to the soft tissue, the bioaccumulation factor of

Cd in the shells was found to be higher by 15 to 30 times. No significant correlation was observed between the concentration of Cd in seawater and soft tissue of mussels, but a strong positive correlation was observed with the level of Cd in the shell. In general, Cd levels in the mussel tissue were markedly lower than those previously reported from the area in green mussels (Krishnakumar *et al.*, 1998 and Sasikumar *et al.*, 2006).

Yap *et al.* (2004b) reported concentration 1.53 ppm in seawater as the LC_{50} value of Cd for *P. viridis*. Cadmium, though highly toxic at lower concentrations, the mean concentrations observed in the seawater of Someshwara (0.0002 ppm) and Surathkal (0.0005 ppm) mussel beds were lower than the LC_{50} reported. The mean tissue concentration of Cd in *P. viridis* collected from Someshwara and Surathkal mussel beds were found to be below the permissible concentration (FDA) given for seafood (WHO 1972, 1987) as well as EU standards for marine products. However, the Cd concentrations in 1% of the green mussels from Surathkal mussel beds slightly exceeded the WHO permissible level for seafood as well as the EU norms (Table 4.11).

Concentration of Pb in seawater was significantly higher at Surathkal but spatial difference in tissue bioaccumulation of lead was not observed between the mussel beds. Pb concentrations observed in *P. viridis* in the present study was low compared to earlier reports of Krishnakumar *et al.* (1998), however, it was more than the levels reported in 2002 from the same area (Sasikumar *et al.*, 2006). Increase in concentrations of Pb in soft tissues of mussels and elevated levels of Pb in seawater samples from Surathkal could be attributed to the input of lead through industrial effluents as well as from petroleum fuel in the vicinity of harbours. Lead is used as a fuel additive and may be released through incomplete fuel combustion and boat bilge discharges (NCDEM, 1991). Pb accumulation observed in green mussels from the mussel beds may be attributed to increase in boating activities. Increase in the concentrations of lead in mussels associated with fuel input from boating activities has been reported in environmental monitoring programmes (Szefer *et al.*, 2004).

The concentration of Pb observed in Someshwara (0.0014 ppm) and Surathkal (0.0019 ppm) mussel beds was lower than the LC_{50} value of 4.20 ppm for *P. viridis* reported by Yap *et al.* (2004b). FDA has set upper limit of Pb concentrations in mussels to 1.7 ppm, when used for human consumption. Pb was not detected in most of the tissue samples from the mussel beds however, in 1% of mussel tissue samples from Someshwara Pb levels slightly exceeded the FDA norms as well as the WHO permissible limit for seafood (Table 4.11). The bioaccumulation of Pb in shell was ten times more than in mussel tissue in both the mussel beds. No significant relationship was observed between the Pb levels in seawater and the mussel tissue and shells.

Nickel was detected in the ambient waters of Someshwara and Surathkal mussel beds. No significant variation was observed with regard to the Ni levels between

the beds. Presence of Ni was identified in the soft tissue of mussels from both the beds and in the shell of mussels from Someshwara. The concentration of Ni recorded in the present study remained within the ranges reported in 2002 (Sasikumar *et al.,* 2006) from the study area and also within the ranges previously reported in green mussel from the southwest coast of India (Radhakrishnan, 1994). With regard to the food safety, the concentrations recorded from the mussel beds are well below the limits set by WHO and US FDA. The bioaccumulation factor of Ni indicated higher values for the mussel tissue than the shells and no significant correlations were detected between the levels of Ni in seawater, tissue and shells.

The estimation of bioaccumulation factor demonstrated that the trace metals are accumulated many folds in the mussel tissue and shells and accumulation of various trace metals do not follow the same pattern. Highest tissue accumulation ratio was noticed for Cu, Cd and Zn in Someshwara and Surathkal mussel beds. Mussel shells showed highest ratios for Cd and Cu, accumulating many folds, more than the ambient water levels. The bioaccumulation level of trace metals was relatively higher at Someshwara in spite of low levels in the ambient waters compared to Surathkal. Overall, the results imply that the mussels accumulate trace metals from the surrounding waters and play a vital role in the transfer of water associated trace metals to higher trophic levels by predation.

4.5.2. Organochlorine pesticides

In the present study, though presence of organochlorine pesticides (OCPs) were occasionally detected in the seawater and mussels from the mussel beds of Someshwara and Surathkal, the concentrations of many of the OCPs were below the maximum residual limits, suggesting that the mussel beds are not significantly contaminated with these substances.

Despite the fact that lower concentrations of DDT were detected in the coastal waters, it was not detected in mussel tissue samples from Someshwara and Surathkal. The ∑DDT concentrations detected in the mussel beds in the present study are less significant compared to those reported in earlier studies from other parts of the Indian coasts (Tables 4.13, 4.14) (Sarkar and Sengupta, 1989 and Pandit *et al.*, 2006)

Higher DDT concentrations were reported from mussels caught from the proximity of urbanized areas in India where, DDT was used for malaria vector control (Tanabe *et al.*, 2000). In the mussel beds, ∑DDT fraction of the OCPs in seawater was comparatively higher at Someshwara than at Surathkal. Variability in the concentration of the residues of different organochlorine pesticides in the coastal areas may be attributed to the river discharge into the coastal waters (Sarkar and Sengupta, 1989). Earlier studies reported that ∑BHC and ∑DDT were the most abundant organochlorine contaminants in sediments near estuarine and offshore regions of Nethravati estuary (Sarkar *et al.*, 1997).

Presumably, there would be a difference in the quality of the environment between the two mussel beds, since the former is potentially exposed to higher concentrations of contaminants through the river discharge and sediment, whilst later would be less influenced by river discharges.

Among the DDT derivatives higher *p,p'*-DDE and *o,p'*-DDD fractions were found at Someshwara than *p,p'*-DDT fraction whereas, at Surathkal only *p,p'*-DDE was detected. Seawater samples from Someshwara, having a DDE/∑DDT ratio of 0.4, had 40% of the ∑DDT existed in the form DDE whereas, at Surathkal only DDE remained indicating the total absence of fresh input of DDT. The detection of very low levels of *p,p'*-DDT in seawater from Someshwara reflects an incidence of inconsequential contamination by DDT, probably attributable to the River discharges. This points out to the fact that DDT is continued to be used in the agricultural treatments, suggesting a relatively recent input at this location, which drains into the river. Residence time of persistent OCPs in the water body appears to be very long and high levels of OCPs have been detected in semi-enclosed coastal areas which receives direct discharges from rivers and industrial/agricultural sources (Wu *et al.*, 1999 and Pandit *et al.*, 2006).

Among the OCPs in mussel tissue, heptachlor epoxide dominated in the samples analysed from Someshwara and Surathkal. Of the cyclodiene organochlorine pesticides (aldrin, dieldrin and endrin), though endrin is relatively short-lived in the environment, it was the major OCP detected in seawater in both the mussel beds, probably reflecting a fairly recent usage. However, endrin levels were below the detectable limits in mussel tissue samples from Someshwara and Surathkal. Though aldrin was detected in seawater from Someshwara, it was below the detectable limits in the water samples from Surathkal. Aldrin, used as a soil insecticide, is the major source of dieldrin (up to 97%) in the environment. Aldrin and the reaction product dieldrin are rapidly adsorbed on soils, especially in soils containing high levels of organic matter. Consequently, there is little penetration into the soil and contamination of ground water does not generally occur. Transport of both compounds takes place mainly through soil erosion (as wind drift) and sediment transport (surface run-off) but generally not through leaching. The occurrence of Aldrin in seawater from Someshwara could be due to inputs from organically rich sediments in Nethravati estuary and run-off from agricultural land. Sarkar *et al.* (1997) detected aldrin from the sediments from almost all the estuaries along the west coast of India, which was attributed to the extensive use of pesticides for agricultural purposes.

Mussels from Surathkal contained comparatively higher concentrations of α-BHC. BHCs' are known to be widely used as insecticides against grasshopper and rice bugs, for seed protection as well as household vector control in India (Li, 1999). Commercial BHCs' are produced mainly in two forms, i.e., a mixture containing α-, β-, γ- and δ-isomers of 55-70%, 5-14%, 10-18% and 6-10%, respectively and lindane of purified γ-isomer. Hence, the predominance of α-

isomer in environmental samples reflects the use of a technical mixture of BHC (Kannan *et al.*, 1995). It should be stressed that ∑BHC both α- and γ-BHC were at low or non-detectable levels in the seawater and mussel tissue at Someshwara, indicating that the contamination was not a result of massive application as pesticides. Earlier studies on the persistent organochlorine compounds in green mussels from the coastal waters of Asian countries including 17 locations along the Indian coasts identified, DDT, α-BHC and PCBs as the predominant organochlorine compound in the tissue (Monirith *et al.*, 2003). In the present study, despite the fact that very low concentrations of ∑BHC and ∑DDT were detected in seawater samples from both the coastal zones of the mussel beds, ∑DDT and γ-BHC were not detected in mussel tissue samples from Someshwara and Surathkal mussel beds. Concentrations of ∑BHC isomers measured in the present study were less than those previously reported in the coastal waters of India (Table 4.13) (Sarkar and Sengupta, 1989 and Pandit *et al.*, 2006).

In general, though the presence of OCPs and its derivatives were detected in the coastal waters of Dakshina Kannada, the concentrations of all compounds studied were very low in the mussel beds of Someshwara and Surathkal. The tissue concentrations of OCPs detected were well below the limits recommended for human consumption. Therefore, the results of this study suggest that the current OCPs levels of the Someshwara and Surathkal mussel beds are unlikely to result in any significant environmental impacts in the near future.

4.5.3. Lysosomal membrane stability

The neutral red retention assay (NRRA) has been demonstrated to be a sensitive indicator of cellular stress level for a range of pollution and contaminant stress in marine molluscs (Lowe and Pipe, 1994; Lowe *et al.*, 1995a,b; Coles *et al.*, 1995 and Cheung *et al.*, 1998). Marine mussels are widely used in both, field and laboratory experiments as sensitive markers of trace metal or organic contamination (Suresh and Mohandas, 1990; Krishnakumar *et al.*, 1994, 2004b; Regoli and Orlando, 1994a,b; Regoli, 1998; Domouhtsidou 2004 and Schiedek, 2006).

Lysosomal system in molluscs accumulates high levels of contaminants and thereby become a specific target of xenobiotics. In the present study, neutral red retention assay indicated that the haemocyte of mussels from Someshwara and Surathkal had the capacity to retain the dye for more than 120 minutes and no difference in the extent of retention time was observed between the two mussel beds. Less retention time is indicative of a higher degree of general stress due to pollution and retention times <60 minutes indicate severely impaired health (Moore *et al.*, 1999). The higher retention time observed in the present study implies that the mussels are not exposed to any considerable contaminant stress induced by the inorganic or organic pollutants. The present observations are in agreement with previous biomonitoring data recorded in other studies where, mussel samples from reference populations in Adriatic Sea retained the dye

between 120 or 180 minutes in *M. galoprovincialis* when, compared to values as low as 0 and 33 minutes from impacted-area (Lowe *et al.*, 1995a).

The biomarker response described corresponded with the tissue burden of contaminants from the mussel beds of the area. The levels of OCPs and trace metals were very low in the mussel beds with concentrations below detectable limit in many of the tissue samples. Furthermore, it is important to consider that the increased retention time probably indicates the absence of a range of other contaminants as well, in the coastal waters. In Karnataka-Kerala coast, Krishnakumar *et al.* (2004b) conducted site-specific integrative environmental quality assessment using biomarkers such as chromosomal aberration, SCE, micronuclei formation, hemic neoplasia and DNA damage in green mussels along with the comparison of the levels of pollutants and observed no indication of stress due to low level of pollution in the region. Ringwood *et al.* (1998) report that neutral red retention is very little affected by temperature or salinity, therefore the observed variations in the CI in both stations had probably no significant influence on the assay result.

The overall picture emerged from the present study indicates that in the Someshwara and Surathkal mussel beds, the trace metal contents in mussel tissue in general are close to background levels. The observations on the trace metal accumulation by mussels are in conformity with the previous reports on trace metals accumulated by bivalve molluscs in the Dakshina Kannada coast and the observed levels do not pose any threat to public health. In general, the mean tissue concentrations of trace metals in *P. viridis* collected from mussel beds of the Dakshina Kannada coast were found to be safe and below the permissible concentrations (FDA) for seafood (WHO, 1972, 1987) as well as EU limits in marine products. The tissue concentrations of the trace metals studied did not present any considerable spatial variations. However, the Cd concentrations in 5% of the green mussels from Surathkal mussel beds and Pb levels in 1% of mussel tissue samples from Someshwara and Surathkal slightly exceeded the WHO permissible level for seafood as well as the EU norms. Thus, the results implicitly suggest the need for regular monitoring of trace metals in the mussel beds to ensure public health safety.

According to the European legislation (EC, 1979) the levels of pollutants in shellfish waters should not exceed those causing deleterious effects on the adult mollusks or their larvae. The LC_{50} values impairing bivalve embryogenesis in marine bivalve species have been reviewed by His *et al.* (2000) and the geometric means are 40 µg/l for Cu, 320 µg/l for Zn, 968 µg/l for Pb and 2219 µg/l for Cd. The concentration of Cu, Zn, Pb and Cd in Someshwara and Surathkal shellfish waters are found to be below these values. Therefore, current levels of these trace metals in the mussel beds do not pose a threat to the bivalve fauna. The observed levels of trace metals in the mussel beds were considerably lower than the LC_{50} values reported for the species (Yap *et al.*, 2004b). The results of the study are indicative of the suitability of the area for development of

a sustainable mussel fishery and culture operations. However, though the metal concentrations in water and mussel tissue were well below the objectionable limits, the situation calls for regular monitoring to ensure that the safe limits are not exceeded. The pollution pressures in the region are likely to increase in the coming years as a result of the fast industrial development and urbanization throughout the coastal zone. This necessitates well designed monitoring plans and concerted efforts to ensure that the pristine coastal ecosystems are protected from pollution effects and consumption of harvested mussels from these waters do not pose any public health hazard.

Table 4.11. Guidelines for metals and organochlorine pesticides in molluscs and seawater.

	Tissue limits (ppm)			**Seawater µg/l (ppm)**
	WHO (1987)	**FDA (2001) (for molluscan bivalves)**	**EC (2001) (for bivalves)**	**EC**
Cadmium	2	4	1.0	2.5 (0.0025)
Copper	30	-	-	5 (0.005)
Lead	2	1.7	1.0	15 (0.015)
Nickel	2	80	-	
Zinc	50	-	-	40 (0.04)
Heptachlor		0.3		
Lindane		0.3		
β-BHC		0.3		
Endrin		0.3		
Aldrin		0.3		
p,p'-DDT		5.0		

Table 4.12. Metal concentrations (ppm wet tissue wt.) reported in green mussel *P. viridis* from west coast of India.

Locations	Cd	Cu	Fe	Mn	Ni	Pb	Zn	Reference
Karnataka								
Karwar ††	0.31±0.08	1.50±0.50		6.80±0.76		0.33±0.10	14.39±0.47	Krishnakumar *et al.* (1990)
Majali††	0.16±0.02	1.77±0.21		5.24±0.33		0.31±0.03	11.06±0.23	
Sungeri Is. ††	0.15±0.03	0.86±0.19		3.62±0.69		0.32±0.03	11.09±1.11	
Devgad Is. ††	0.34±0.08	2.25±0.32		6.98±0.14		0.50±0.07	14.54±0.52	
Arga††	0.22±0.08	1.57±0.63		8.86±0.20		0.80±0.08	13.97±2.89	
Amdalli††	0.28±0.04	2.89±0.67		6.18±0.74		0.22±0.06	13.93±0.25	
Harwada††	0.24±0.03	2.62±0.19		8.10±0.73		1.03±0.42	14.16±1.10	
Belekeri††	0.12±0.06	1.86±0.21		6.67±0.24		0.26±0.03	11.62±0.98	
Majali††	0.16	1.8				0.31	11.1	Krishnakumar *et al.* (1998)
Karwar††	0.31	1.5				0.33	14.4	
Argae††	0.22	1.57				0.8	14	
Kaup††	0.54	17.1				1.48	23.3	
Surathkal††	0.71	23.6				1.8	49.7	
Thaneerbhavi††	0.81	128				2.52	70.5	
Karnataka ††	0.03- 3.71	nd –2.3	nd – 285.4	nd – 11.4	nd – 3.5	nd – 2.00	nd – 34.1	Sasikumar *et al.* (2006)
Kerala								
Cochin †		22.26±5.5	374.6±257.7			7.59±1.27	79.18±12.14	Lakshmanan and Nambisan (1983)
Calicut†	1.21±0.57	12.50±8.70	352.5±103.4		2.11±1.32	3.89±1.11	93.39±79.44	Pillai *et al.* (1986)
Calicut & Cochin†	2.5	7.34	16.28	0.33	2.25	6.12	344.52	Radhakrishnan (1994)
Calicut††	0.1	6.5		2.8	0.8		15.7	Sankar et al. (2006)
Gujarat								
Mocha†	1.53	3.91	9.40	1.01		20.10	37.34	Tewari *et al.* (2000)
Mocha†	4.2-1.08	6.82-21.22	230.6-302.3	5.25-16.28	19.5-53.15	30.56-63.84	40.23-82.8	Tewari *et al.* (2001)
Dwaraka†	2.54-9.48	14.24-40.47	389.4-618.3	12.52-29.54	32.81-65.45	45.28-92.38	52.62-813.28	
Someshwara††	0.03±0.10	3.38±1.97	30.56±42.4		0.041±0.17	0.11±0.84	14.07±5.28	Present study
Surathkal††	0.10±0.46	2.98±3.07	33.6±46.6		0.26±1.64	0.06±0.48	14.94±7.21	Present study

† Dry wt. †† Wet wt.

Table 4.13. Organochlorine pesticide concentrations reported in green mussel *P. viridis* and seawater from west coast of India.

Mussel (ppb wet wt.)																
Location	**α-BHC**	**β-BHC**	**γ-BHC**	**Heptachlor**	**Aldrin**	**Heptachlor epoxide**	**Dieldrin**	**Endrin**	***o,p'*-DDT**	***p,p'*-DDT**	***p,p'*-DDE**	***o,p'*-DDE**	***o,p'*-DDD**	***p,p'*-DDD**	**Total**	**Authors**
Munambam †	0.003		0.001	0.001	0.003	0.007	0.002	0.017	0.013	0.038	0.006		0.005		0.095	Radhakrishnan *et al.* (1986)
Calicut†	0.050		0.002	0.003	0.013	0.004	0.002	0.002	0.010	0.013	0.001		0.006		0.106	Radhakrishnan *et al.* (1994)
Munambam†	0.052		0.002	0.003	0.001	0.004	0.002	0.002	0.010	0.014	0.001		0.006		0.096	Radhakrishnan *et al.* (1996-unpublished)
Calicut						0.060								0.160	0.220	Shankar *et al.* (2006)
Someshwara	0.008					0.424									0.431	Present study
Surathkal	0.108				0.102	0.326									0.536	Present study
Seawater (ppb)																
Central west coast	0.26-9.4				nd-9.8		nd-50.9		nd-251	nd-56	nd-20.34	nd-64.5	nd-137			Sarkar and Sen Gupta (1989)
Mumbai	1.230	1.410	4.710							8.520	0.870				16.740	Pandit *et al.* (2006)
Someshwara	0.021		0.0001	0.001	0.003	0.005		0.066		0.006	0.029		0.032		0.164	Present study
Surathkal	0.022		0.0001					0.052			0.007				0.081	Present study

† **in ppm**

Table 4.14. Organochlorine pesticide concentrations (ppb wet wt.) reported in green mussel *P. viridis* from west coast of India.

Location	**∑DDT**	**∑BHC**	**Authors**
Munambam-SW-India†	0.062	0.004	Radhakrishnan *et al.* (1986)
Calicut-SW-India†	0.029	0.052	Radhakrishnan *et al.* (1994)
Munambam-SW-India†	0.03	0.054	Radhakrishnan *et al.* (1996-unpublished)
South India	3.0-40	4.3-16	Ramesh *et al.*, (1990)
India	0.90-40	1.5-12	Kan-atireklap *et al.* (1998)
India	0.60-15	0.20-7.7	Monirith *et al.* (2003)
Calicut-SW-India	0.16	nd	Sankar *et al.* (2006)
Someshwara-SW-India	nd	0.008	Present study
Surathkal-SW-India	nd	0.108	Present study

† in ppm;
nd - below detectable limits

Chapter 5

Sanitary quality of shellfish waters

5.1. Introduction

Organic pollution of the overlaying coastal waters of the mussel beds invites special attention owing to the public health concerns of the harvested mussels. The significance of the harvest/culture area where mussels are grown, on the quality of the mussel arises from the unique feeding behaviour of bivalves. The filter feeding mechanism in mussel, the lack of specificity and selectivity in the filtration process together with their ability to filter significant quantities of water relative to their size render them as potential vectors of water born pathogens even in the size range of bacteria and viruses (Ayres *et al.*, 1975).

It is reported that a single mussel filters up to 4.9 l of seawater per gram body weight in one hour (Gardner, 2002). In this process they may ingest and accumulate pollutants, pathogenic microorganisms and naturally occurring toxins in their digestive system and tissues from the surrounding waters. Therefore, the public health concerns will continue to play an important role in molluscan aquaculture development and product marketing (Shumway, 1992).

The sanitary quality of the mussel growing waters is extensively used in many countries for ascertaining the quality of the mussel. Mussel beds are by and large situated in shallow near-shore and estuarine waters exposing the mussels to contaminants present in land or river run-off or direct sewage discharge which are the major sources of microbial pollution. If exposed to a considerable load of pathogens over certain period of time, the pathogens are accumulated in their soft tissues at levels hazardous to the consumers, especially when it is consumed raw or in partially cooked form.

This risk of harvesting mussels from polluted waters increases with the proximity of mussel beds to highly urbanized or agricultural areas. The pollution conditions are often aggravated by rainfall resulting in sewage-contaminated run-off or effluent from overloaded sewage treatment systems reaching the mussel beds.

The major sources of microbial pollution are domestic sewage (treated and untreated), river runoff, land runoff, treated and untreated industrial wastes, sewage disposed from mooring boats on the beaches and recreational activities on the beaches. Besides this, the water quality of rivers discharging into the coastal area also influences the microbial levels of the inshore waters. The quality of river water is in turn influenced by the inputs derived from human activities such as agriculture, animal husbandry and also from non-point sources. Surface water canals, rivulets and streams and surface runoffs from these areas carry animal wastes, manures and silt into the river and finally to the estuary.

Microorganisms of major concern in mussel sanitation are bacteria and viruses. Blue mussel (*Mytilus edulis*) has been investigated by bacteriologists over many decades for pathogenic bacteria (al-Jebouri and Trollope, 1981) of public health significance. When mussels are exposed to faecal contamination from domestic sewage discharges, they are often subjected to pathogenic bacteria (Wood, 1972). Some of the important pathogenic bacteria of public health significance reported from shellfish grown in polluted waters belong to the genera, *Salmonella, Vibrio* and *Clostridium* (Shumway, 1992). Besides these, bacteria which are part of the normal fauna of human digestive system can cause problems if ingested in excessive numbers. Further a wide range of viral pathogens of terrestrial animal origin are also likely to be present in both raw and treated sewage and poses threat of their transfer through contaminated shellfish.

The objective of sanitary control of shellfish is to ensure that these pathogenic organisms are either absent or present only in numbers below acceptable limits in the harvested mussels. Since many pathogens associated with faecal matter are discharged into coastal waters, the demonstration of pathogenic bacteria would obviously constitute the most direct proof of a dangerous impurity. However, these organisms, particularly the pathogens, are difficult to enumerate when, present in very low concentrations and the difficulty of their isolation makes the test impractical for ordinary purposes. Consequently, estimates of accumulation of contaminants (bacteria and viruses) are based on indicator organisms, which indicate that faecal matter has entered the water body and that the water is therefore liable to contamination with pathogenic organisms.

To be satisfactory, an indicator organism must always be present when the pathogenic organisms are likely to be present, but not when they are absent. It must be excreted in much greater numbers than the pathogens and must have a comparable resistance. It should not be able to proliferate to any greater extent

than pathogens in aquatic environment. To be acceptable, it should be capable of identification and enumeration by simple techniques in a reasonably short period but unfortunately no organism meets all these criteria. The best indicator would obviously be the one whose density correlates best with the health hazards associated with faecal contamination. Among indicator organisms, the members of the coliform group have received most attention. Because of the wide distribution of some members of the coliform group in natural unpolluted waters, the faecal coliforms are used as an indicator of a breach of water quality since the turn of the century.

Faecal coliforms which comprise a portion of the total coliform group have proven to be of more sanitary significance because they are restricted to the intestinal tract of the warm blooded animals and are consequently used to define water quality and sewage pollution. Few faecal coliforms are pathogenic however, the presence of faecal coliform bacteria in coastal waters indicates faecal pollution from warm-blooded animals.

Among faecal coliforms most notably the *Escherichia coli* are used as indicator organisms. *E. coli* occurs in abundance and reflects in a relative way the possible concentrations of pathogens from sewage as they are the standard accepted indicator of faecal contamination (Bernard, 1989). Enumeration of the coliform group as a whole and *E. coli* in particular, is used to indicate the level of pollution in seawater and in the mussel, as it has a lower survival rate in seawater. Therefore, current measures for controlling public health problems associated with the consumption of sewage-contaminated shellfish rely on the use of *E. coli* to determine the sanitary quality of shellfish harvesting areas (Lees, 2000). Since the *E. coli* is derived from the intestines of warm blooded organisms, they are likely to occur in small numbers even in water bodies far removed from the possibility of human contamination. Water grossly contaminated with sewage contains them in large numbers. Therefore, test for their presence as an index of the degree of pollution must be carried out on a quantitative basis.

Sewage pollution of shellfish growing waters is a world wide problem which is continuously aggravated by increasing human population (Ray and Rao, 1984). However, the point-sources of these types of contaminants are generally easily identified and monitoring programmes can be developed to predict the risk to the shellfish consuming public.

To ensure that the sewage organisms do not reach the consumer, the seawater from which the shellfish are harvested or the product or both can be examined. The use of seawater is attractive since sampling and examination of the water is relatively simple, but at best this method can be regarded as an indirect way of assessing the pollution of an area and the quality of shellfish. Even then, this requires continuous monitoring of the seawater for possible contamination. Consequently various protocols for assessing the level of sewage contamination

of molluscs for controlling their exploitation from polluted areas have been developed by many countries.

In many countries the shellfish monitoring departments routinely monitor faecal coliform and water quality parameters and have enacted sanitary controls on the production of live bivalve molluscan shellfish. Presently, water quality standards to measure the sanitary quality of the shellfish growing waters are rigidly enforced in many countries and shellfish growing waters are approved prior to the harvesting of shellfish for human consumption. The major objective of such shellfish water quality protection programme is to protect the public from the consumption of contaminated shellfish by ensuring that bivalves are harvested from waters of acceptable sanitary quality and also to promote pollution prevention, remediation and restoration of contaminated bivalve growing areas.

In the European Union, shellfish quality assurance is covered by Council Directive 91/492/EEC (EC, 1991) and in the United States, by interstate trading agreements set out in the FDA National Shellfish Sanitation Program Manual of Operations (NSSP, 1999). The European community has pronounced the gram-negative bacterium *E. coli* as indictor organism for faecal contamination in bivalves. EEC regulation 79/923 deals with the classification of waters used for shellfish farming and EEC regulation 91/492 deals with the definition of health standards applicable to the production and sale of live shellfish. The regulation takes into account only the number of faecal coliforms and *E. coli* in waters and shellfish.

Classification of harvesting areas for shellfish in the EU, microbiological examination of shellfish samples is based on the *E. coli* and/or faecal coliform count of shellfish within the harvesting area (EC, 1991). It is classified into class A (<230 *E. coli*/100g or <300 faecal coliforms/100g) requiring no treatment prior to consumption, class B (<4,600 *E. coli*/100 g or <6,000 faecal coliforms/100g in 90% of the samples) with shellfish requiring depuration or relaying and class C (<60,000 faecal coliforms/100 g) with shellfish requiring relaying over a long period in clean seawater until they meet class A standards.

The US FDA National Shellfish Sanitation Program (NSSP, 1999) similarly rely on faecal coliform monitoring of harvest waters in order to determine the approved and restricted harvest areas and treatment requirements prior to sale. Shellfish growing waters approved for unrestricted shellfish harvest may have a highest mean faecal coliform MPN of 14/100 ml, with 10% of the samples not exceeding an MPN of 43/100 ml in a five-tube decimal dilution test. US FDA "approved" and/or EU class-A harvest sites are the cleanest growing areas from which shellfish can be drawn for human consumption without further processing. Shellfish from EU class-B and US FDA restricted harvest areas may only be placed on the market following depuration or relaying.

In Australia, shellfish growing waters are classified based on the Australian Shellfish Quality Assurance Programme (ASQAP, 2004) developed in line with

US NSSP. A Similar scheme of classification based on US NSSP is established in New Zealand, known as the New Zealand Shellfish Quality Assurance Programme (NZSQAP) which classifies all commercial shellfish growing areas in New Zealand.

In India, though bivalves are not consumed raw, adherence to recognised microbiological guidelines is essential for facilitating access to overseas markets and to ensure that only safe and wholesome shellfish is marketed. The formulation of policies, regulations and establishment of facilities to ensure the safety of this seafood, which has the possibility of contamination by bacterial or viral pathogens, is very urgent and essential for the growth and development of the shellfish industry. In the country, however rigorous may be the aquaculture promotion activities, trading partners cannot be established unless international regulatory standards of food safety are met and accepted. Comprehensive, standardized shellfish sanitation programmes are therefore a priority requirement. Moreover, increased global concern has resulted in rigid shellfish safety programmes in more and more countries and trading partnerships. Programmes regulating exports to the European Union and the United States are of particular importance in this regard. Strict regulations have to be enacted, necessitating extensive monitoring and certification that the seafood is free from bacteria and other biological and chemical substances that are harmful to humans. Monitoring programmes that use approved procedures have to be implemented with overview by accredited and competent authority. Shellfish safety monitoring should be implemented in the country on a priority basis as it is envisaged to give thrust to the development of mussel culture and/or harvest activities in the near future aiming the potentially lucrative export markets for high quality shellfish products.

The mussel beds off Someshwara and Surathkal along the Dakshina Kannada coast are influenced by the inputs derived from land use and human activities in the surrounding region from point and non-point sources. Major occupations along the coastal areas of Dakshina Kannada are agriculture, fishing and fish processing. Besides, the direct input of organic wastes from point and non-point sources in the vicinity, the mussel beds are subjected to organic loading brought in by the Nethravati, Gurpur, Pavanje and Sambhavi Rivers flowing to the nearby Nethravati-Gurpur and Mulki estuaries. Water quality of these rivers is largely influenced by the inputs derived from land use which is predominantly agriculture and other human activities in the river banks and the catchment areas.

A comprehensive study on the sanitary quality of mussel harvesting areas of Dakshina Kannada District is lacking. Present investigation was carried out to evaluate the sanitary quality of the mussel beds of the District and for collecting baseline information on the extent of faecal contamination of mussel beds. The results of the study will provide valuable information on the present status of mussel beds with respect to the sanitary standards. Such area specific studies also could provide inputs in establishing national regulations for the sanitary

control of shellfish industry. Biological Oxygen Demand (BOD) of the coastal waters of mussel beds was also investigated to evaluate the extent of organic loading.

The information on the seasonal variations in the presence of indicator organisms in the mussel beds and the status of the shellfish waters will be useful for an effective management of coastal areas of the District which is currently experiencing intense urbanisation pressure. The study was carried out with this objective of appraising the sanitary quality of the shellfish waters and the mussel.

5.2. Review of Literature

The sanitary standards of the mussel beds have been widely studied, primarily for assessing the health risks associated with the consumption of bivalve shellfish. Faecal coliforms are commonly used as indicator organisms to signal the possible presence of faecal and pathogenic organisms and for evaluating the quality of the environment.

Accumulation and elimination of coliforms by shellfish have been detailed in many studies. Cabelli and Heffernan (1970) studied the environmental parameters significant to the accumulation of bacterial pathogens by shellfish grown in polluted waters and discussed the kinetics of the uptake process. Canzonier (1971) monitored the *E. coli* uptake and elimination by the hard clam, *Mercenaria mercenaria*, simultaneously with the accumulation and elimination of viral particles with the coliphage S-13 as a working model. Son and Fleet (1980) reported the behaviour of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, relaying and storage. The relationship of faecal coliforms, *E. coli* and *Salmonella* spp. was examined in freshly harvested and stored shellfish by Hood *et al.* (1983) and suggested that low faecal coliform levels in both fresh and stored oysters are good indicators of the absence of *Salmonella* spp., but that high levels of faecal coliforms are limited in predicting the presence of *Salmonella* spp. Burkhardt *et al.* (1992) examined the effects of season and temperature on the ability of *M. mercenaria* to accumulate both faecal coliforms and other sanitary indicator organisms (*E. coli*, *Clostridium perfringens* and male-specific bacteriophages). Burkhardt and Calci (2000) investigated the ability of eastern oyster (*Crassostrea virginica*) to accumulate indicator microorganisms (faecal coliforms, *E. coli*, *C. perfringens* and F1 coliphage) from estuarine waters. Hernroth *et al.* (2002) investigated the influence of environmental factors such as temperature, salinity and land runoff on the variability of enteric viruses and their potential indicator organisms in mussels. Shieh *et al.* (2003) carried out a survey to examine the prevalence of human enterovirus and Norwalk-like virus in environmentally polluted shellfish to assess shellfish safety with the focus on viral contamination and to evaluate their relationship with microbial indicators. Cruz *et al.* (2003) analysed the distribution of faecal viral pathogens in shellfish samples under diverse conditions in diverse geographical areas. Dore *et al.* (2003) worked on the levels of male-specific RNA bacteriophage and *E. coli* in bivalves collected from commercial harvesting areas inorder to introduce F-RNA bacteriophage as an indicator of the viral risk associated with shellfish. Marino *et al.* (2005) investigated the uptake of *E. coli*, *Vibrio cholerae* and *Enterococcus durans* and depuration by mussel *Mytilus galloprovincialis* inorder to determine the most useful indicator of vibrio contamination. Mujika *et al.* (2002) studied the depuration dynamics of viruses in shellfish and suggested 5-day depuration treatment under technically well-controlled conditions in order to ensure elimination of viruses in mussels.

Richards (1978) compared methods for the enumeration of total and faecal coliforms in the eastern oyster, *C. virginica.* Grabow *et al.* (1992) compared different methods for the enumeration of faecal coliforms and *E. coli* in naturally contaminated and sewage-seeded mussels and oysters. Cruz *et al.* (2002) while validating reliable techniques for the detection and quantification of human enteric viruses in shellfish from diverse geographical areas analysed the presence of *E. coli.* The history, application and methodology for the most commonly used indicator organisms including *E. coli* in assessing the microbial status of water and food is summarised by Tortorello (2003).

Edmonds (1976) studied the survival of coliform bacteria in sewage sludge and the potential movement into groundwater. Anderson *et al.* (1979) studied the sublethal stress in *E. coli* in various test media after exposure (*in vitro*) to seawater of various salinities and observed greater stress at higher salinity. Goyal (1979) assessed the nature, extent and sanitary significance of enterovirus pollution in the waters and shellfish and compared with various physico-chemical characteristics and bacteriological quality of the water. Labelle *et al.* (1980) correlated the number of viruses in sediments with various bacterial indicators in sediments which receive domestic sewage. Hood and Ness (1982) examined the survival of strains of *V. cholerae* in estuarine waters and sediments and compared their survival to that of *E. coli.* Anderson *et al.* (1983) investigated the seasonal variation in survival of *E. coli* exposed *in situ* in membrane diffusion chambers containing filtered and non-filtered estuarine water. Kenyon *et al.* (1984) reported direct relationship between the recovery of *V. cholerae* with total number of coliforms in coastal waters. Colley *et al.* (1994) investigated inactivation of enterococci and faecal coliforms by sunlight in sewage effluent diluted in seawater in field experiments. Sinton (1994) outlined the interacting factors (nutrient availability, salinity, temperature, pH, microbial predation and solar radiation) affecting the survival of faecal indicator bacteria in seawater and reported the importance of solar radiations. Davies *et al.* (1995) studied the survival of faecal coliforms, faecal streptococci and *C. perfringens* spores in freshwater and marine sediments from sites near sewage outfalls. Legnani *et al.* (1998) studied the microbiological quality of shellfish waters and examined the relationship between the traditional bacterial indices for faecal contamination and other indicators of microbiological quality. Sinton (1999) compared sunlight inactivation rates of somatic coliphages, F-specific RNA bacteriophages (F-RNA phages) and faecal coliforms in seawater. Tree *et al.* (2003) studied the effect of chlorination on the inactivation rates of indicator bacteria and viruses in primary treated effluents. Neill (2004) developed microbiological indices for assessing the bacterial counts of total coliform and *E. coli* in estuarine waters. Anderson *et al.* (2005) measured persistence of indicator organisms by decay rates (change in culturable counts over time) and reported that the decay rates are influenced by the indicator organisms, inoculum, water type, sediment versus water column location and *E. coli* strain. Faecal coliform decay rates were significantly lower than those of enterococci in freshwater but were not significantly different in saltwater.

Along the west coast of India, published reports on the sanitary quality of the shellfish growing waters are very few. Venkataraman and Srinivasan (1955) studied the *E. coli* levels in *P. viridis* of Korapuzha Estuary near Calicut, along the southwest coast of India. The study showed the presence of *E. coli* in the waterbody throughout the year with peak pollution immediately following the beginning of the southwest monsoon. Gore and Singbal (1973) made qualitative and quantitative studies on the Enterobacteriaceae (coliforms) of the sandy beaches of Goa. Gore *et al.* (1980) examined the coliform counts in sediments and seawater while isolating *Salmonella* spp. from the beaches of Kerala and reported high faecal pollution with high *E. coli* counts. Pillai (1980) observed the levels of coliforms in brown mussel *P. indica* in cultured as well as wild stocks at Vizhinjam along the southwest coast of India. Row (1981) presented an account on the distribution and monthly variation of total heterotrophic bacteria and coliforms in Mandovi and Zuari estuaries. They observed relatively high coliform counts in the coastal waters off Mandovi-Zuari estuary during September-October months as compared to low levels during the rest of the year and attributed this to the recent flushing of the estuarine waters into the sea. Selvan and Pillai (1988) studied the bacterial quality of brown mussel *Perna indica* from natural beds. Surendran and Balachandran (1988) examined the faecal coliforms and faecal streptococci levels in mussel *P. viridis* from the Vembanad lake and described the cleansing procedure for elimination of pathogenic/indicator organisms from the mussel. Bacterial contamination of the beach sand, water and mussel from Mahe was investigated by Gore *et al.* (1992). Brock *et al.* (1985) developed simple linear models on the relationships of rainfall, river flow and salinity with the faecal coliform levels in mussel beds. Surendran *et al.* (2002) while investigating the microbial pollution in *P. viridis* from the brackish water bodies of Calicut, enumerated the levels of coliforms. Ramaiah *et al.* (2004) quantified the abundance of pollution indicator and pathogenic bacteria in Mumbai waters.

Information on the sanitary quality of the mussel growing or harvest waters along the Karnataka coast with regards to the seasonal variations in the indicator organisms in mussel beds are limited. Studies on the detection and isolation of pathogens from estuarine shellfish of Karnataka coast have been carried out by Karunasagar *et al.* (1987, 1990, 1995, 1996), Dileep *et al.* (2003) and Parvathi *et al.* (2005). Sunil *et al.* (2004) analysed the bacteriological quality of water and sediment samples collected from Nethravati and Sharavathi Rivers as well as from the adjacent estuarine and sea sites. Deepanjali *et al.* (2005) reported the faecal coliform counts in oysters collected from two estuaries along the southwest coast of India while, studying the seasonal abundance of *Vibrio parahaemolyticus*.

5.3. Materials and Methods

5.3.1. Sampling

Samples of seawater, sediment and mussels were collected on a monthly basis during January - December 2003 from the two mussel beds situated off Someshwara and Surathkal. At each mussel bed, six sampling points were fixed along the coastline covering the area of peak fishing activity. The sampling points were fixed at equidistant interval of 100 m parallel to the coast line and at a depth of 3-4 m.

Water samples were drawn from the surface layer (<50 cm) using 100 ml sterile PE bottles. Mussel and sediment samples were also collected from the same sampling points and kept in sterile bags. The samples were transported to the laboratory in insulated box and were processed immediately (within 5-6 h from time of collection).

A total of 93 seawater samples, 84 sediment samples and 96 mussel samples were collected from Someshwara and 112, 105 and 117 seawater, sediment and mussel samples respectively were collected from Surathkal for analysis.

5.3.2. Biochemical Oxygen Demand (BOD)

Water samples for analysing the BOD were collected in BOD bottles from the selected sampling points. Initial DO levels were measured and samples were transported to the laboratory in insulated box. The samples were incubated without dilution at 20°C for 5 days. The BOD_5 was estimated following Strickland and Parsons (1972).

5.3.3. Microbiological examination techniques

5.3.3.1. Media and reagents

The bacteriological media and reagents used for the sample preparation and enumeration of total coliforms, faecal coliforms and *E. coli* are detailed below:

Physiological saline

Sodium chloride : 8.5 g
Distilled water : 1000 ml
Sterilised by autoclaving at 121°C for 15 minutes.

Peptone water 0.1%

Peptone : 1 g
Distilled water : 1000 ml
pH : 7.0±0.2
Sterilised by autoclaving at 121°C for 15 minutes.

Confirmed Phase – Total coliforms

Brilliant Green Lactose Bile (BGLB) Broth (HIMEDIA, Mumbai)

Peptone	: 10.0 g
Lactose	: 10.0 g
Oxgall	: 20.0 g
Brilliant green	: 0.0133 g
Distilled water	: 1000 ml
pH	: 7.2±0.2

Medium was prepared as per the manufacture's instruction.

Confirmed Phase – Faecal coliforms

***Escherichia coli* (EC) Broth** (HIMEDIA, Mumbai)

Tryptose	: 20.0 g
Lactose	: 5.0 g
Bile salts mixture	: 1.5 g
Dipotassium hydrogen phosphate	: 4.0 g
Potassium dihydrogen phosphate	: 1.5 g
Sodium chloride	: 5.0 g
Distilled water	: 1000 ml
pH	: 6.9±0.2

Medium was prepared as per the manufacture's instruction.

Confirmed Phase – *E. coli*

Eosin Methylene Blue (EMB) Agar (HIMEDIA, Mumbai)

Peptone	: 10 g
Lactose	: 10 g
Dipotassium phosphate	: 2 g
Eosin Y	: 0.4 g
Methylene blue	: 0.065 g
Agar	: 15 g
Distilled water	: 1000 ml
pH	: 7.1±0.1

Medium was prepared as per the manufacture's instruction.

Tryptone Broth (HIMEDIA Mumbai)

Tryptone	: 10 g
Yeast extract	: 5 g
Sodium chloride	: 5 g
Distilled water	: 1000 ml
pH	: 7.1±0.2

Medium was prepared as per the manufacture's instruction.

Kovac's Reagent (HIMEDIA, Mumbai)

p-Dimethy amine benzaldehyde	: 5.0 g
Isoamyl alcohol	: 750 ml
Conc. Hydrochloric acid	: 250 ml

Methyl Red-Voges Proskauer Broth

Glucose	: 5.0 g
Peptone	: 7.0 g
Dipotassium hydrogen phosphate	: 5.0 g
Sodium chloride	: 5.0 g
Distilled water	: 1000 ml

Medium was dispensed in 5 ml proportion into tubes and autoclaved at 110°C for 15 minutes

Methyl Red reagent

Methyl red	: 0.2 g
Ethyl alcohol	: 600 ml

Reagent was made up to 1000 ml with distilled water.

Voges Proskauer's reagent

Solution A

α-naphthol	: 5.0 g
Absolute alcohol	: 100 ml

Solution B

Potassium hydroxide	: 40% strength

Simmon Citrate Agar (HIMEDIA, Mumbai)

Sodium citrate	: 2.0 g
Sodium chloride	: 5.0 g
Dipotassium hydrogen phosphate	: 1.0 g
Ammonium hydrogen phosphate	: 1.0 g
Magnesium sulphate	: 2.0 g
Bromothymol blue	: 0.08 g
Agar	: 15.0 g
Distilled water	: 1000 ml
pH	: 6.8±0.2

Medium was prepared as per the manufacture's instruction, dispensed into tubes, sterilized and solidified in a slanting position.

5.3.3.2. Most Probable Number (MPN) technique

Seawater and mussel samples were analysed for total coliforms, faecal coliforms and *E. coli* using the MPN technique in accordance with the statutory method proposed by NSSP (1999). The samples from the six independent sampling points of the two mussel beds were analysed separately.

The MPN procedure involves a multiple tube fermentation technique where, three or more decimal dilutions of the sample are inoculated into tubes containing bacteriological media and incubated at a specific temperature for a specific time. The method has been shown to produce satisfactory results with naturally contaminated foods and water for the detection of coliforms, faecal coliforms and aerogenic *E. coli* (APHA, 1984). Briefly, the method involves serially diluting the target microorganisms in the sample, in 5-replicate aliquots

to extinction. The probable level of the target organisms is then statistically estimated from an MPN Table. Gas production is used as an indication of ability to ferment lactose from Lauryl tryptose (LST) broth (presumptive media); gas production from Brilliant Green Lactose Bile (BGLB) broth is considered confirmation of coliform presence. Gas production at 44.5 or 45°C from EC broth is used as confirmation of faecal coliform presence. Appearance of typical nucleated, dark-centred colonies with metallic sheen when positive EC broths are streaked onto L-EMB agar are indicative of *E. coli*. The typical colonies on L-EMB agar must be confirmed by Indole, Methyl red, Voges-Proskauer, Citrate (IMViC) biochemical tests to prove the presence of *E. coli*. Based on the number of tubes indicating the presence/ absence of the three groups of organisms, the most probable numbers present is estimated from a standard MPN Table.

The total coliforms, faecal coliforms and *E. coli* in water, sediment and mussel samples were enumerated using 5 tube MPN procedure. In case of water samples, 10 ml, 1 ml and 0.1 ml portions were used for inoculation. Sediment samples of decimal dilution were obtained by mixing 50 g with 450 ml of 0.1% peptone water. The mixture was allowed to settle and the supernatant liquid was used for inoculation (APHA, 1995). Mussel samples were analysed immediately after collection. Ten to 12 live mussels were randomly selected to obtain a minimum of 200 g of meat and liquor. The mussel tissue samples were prepared according to standard method (NSSP, 1999). The collected meat and liquor was weighed, blended, diluted (decimal dilution using 0.5% peptone water) and inoculated into tubes of presumptive media (LST broth). A set of five tubes containing 10 ml of double strength LST broth and two sets of five tubes containing single strength LST broth were serially inoculated with 10 ml, 1ml and 0.1 ml portions of the samples. The tubes were incubated at 37±0.5°C for 24±2 h along with an uninoculated tube as control and were examined for gas formation and the results were recorded. Development of turbidity with gas formation in LST broth constitutes positive result for the presumptive coliform test.

5.3.3.3. Enumeration of total coliforms

All positive tubes in LST broth were selected for the confirmation of coliforms, faecal coliform and *E. coli*. The gas-negative tubes were incubated (except raw mussel tissue) for an additional 24±2 h and the numbers of positive results were appended to result obtained after 24 h. Absence of gas in all of the tubes after 48 h (24 h for raw mussel tissue) of incubation indicates a negative presumptive result. Brilliant Green Lactose Bile (BGLB) broth was used for the confirmation of the total coliforms. The BGLB broth tubes prepared were inoculated with inoculum from the positive LST tubes. The positive LST broth tubes were rotated to mix the contents and one loopful from each tube was transferred to a tube of BGLB broth using a sterile inoculation needle. The inoculated medium was mixed by gently shaking the tubes without entrapping air in the gas vials. The inoculated BGLB broth tubes were incubated at 37±0.5°C for 24±2 h and

examined for gas formation and the results were recorded. The negative tubes were incubated for an additional 24±2 h, re-examined and the results were added to the results obtained in 24±2 h incubation. MPN of confirmed total coliforms was computed by converting the number of gas-positive tubes to MPN values per 100 ml for water and 100 g for sediment and tissue.

5.3.3.4. Enumeration of faecal coliforms

Media used for the confirmation of faecal coliforms was *E. coli* (EC) broth. Gas production with growth in an EC broth within 24±2 h or less is considered as positive faecal coliform reaction. The contents of the positive BGLB broth tubes were mixed by rotating the tubes and one loopful from each was transferred to a tube of EC broth. The inoculated EC broth tubes were incubated in a water bath at 44.5±0.5°C for 24±2 h. The tubes were examined for gas production (gas bubble or effervescence) and the results were recorded. Formation of gas during 24±2 h incubation constitutes a positive faecal coliform test. MPN of confirmed faecal coliforms was computed by converting the number of gas-positive tubes to MPN values per 100 ml for water and 100 g for sediment and tissue.

5.3.3.5. Enumeration of E. coli

Eosine Methylene Blue (EMB) agar was used as selective medium for the isolation of *E. coli*. The colonies of *E. coli* appear as non-mucoid, nucleated, dark-centered colonies with a metallic sheen on EMB agar. For isolation of *E. coli,* a loopful of the culture from gas-positive EC broth tube was streaked on an EMB agar plate. The inoculated plates were incubated at 37°C for 18 to 24 h and examined for typical colonies which are indicative of *E. coli*. The typical *E. coli* colonies were subcultured on PCA slants and incubated at 37°C for 18-24 h. Further confirmation of *E. coli* was done by the IMViC tests.

IMViC tests

The characters of *Escherichia* are that it produces indole, gives positive methyl red reaction and a negative Voges-Proskauer reaction and does not utilize citrate.

Indole (I): Inoculum from colonies identified on EMB plate was transferred to a Tryptone broth and incubated at 37°C for 48±2 h. Kovac's reagent was added to the culture for the confirmation of indole production. Formation of a dark red ring in the surface layer indicates a positive reaction and a yellow colour indicates negative reaction.

Methyl-Red (MR) and Voges-Proskauer (VP) Tests (MVi): *E. coli* gives a positive reaction in MR, indicated by the development of red colour and a negative reaction in VP test characterised by the absence of any colour development within 5-10 minutes. The MRVP test was performed by inoculating two tubes of MR-VP broth with inoculum from colonies isolated on PCA slants. The inoculated tubes were incubated at 37°C for 48±2 h. Few drops of methyl-

red solution were added to one set of tubes. To 1 ml of 48 h culture medium, 0.6 ml of naphthol solution and 0.2 ml 40% KOH were added followed by a few crystals of creatine, shaked and allowed to remain for 2 h. Positive result is indicated by the development of eosin pink colour.

Simmon's Citrate Test (C): In this test the ability of cultures to utilize citrate as a source of Carbon is tested on Simmon Citrate (SC) agar slants. Positive reaction is indicated by visible growth, usually accompanied by a change of colour from green to deep blue. *E. coli* on SC slants provide a negative reaction. The test was performed by inoculating SC agar slants by applying a light inoculum from colonies isolated on PCA slants using a straight needle. The slants were incubated at 37°C for 48±2 h and results were recorded.

The MPN for *E. coli* in water, sediment and tissue was computed based on proportion of EC tubes in 3 successive dilutions that contain *E. coli* as detailed for coliforms above.

5.3.4. Evaluation of sanitary standards of mussel beds

Various shellfish sanitation strategies have been implemented in shellfish growing areas throughout the world which include a system of classification of shellfish growing areas broadly based on either water test results (USA and Canada-National Shellfish Sanitation Program, NSSP, 1999); Australia-Australian Shellfish Quality Assurance Program (ASQAP, 2004)) or tissue test results (Europe-Council Directive 91/492/EEC, EC, 1991) combined with the use of relaying or controlled depuration.

The status of the sanitary quality of the mussel beds were evaluated based on the geometric mean of the faecal coliform levels in shellfish growing waters as per US FDA NSSP (NSSP, 1999) and the mussel beds are classified accordingly. The 90^{th} percentile of the geometric means were used to identify threatened areas as it responds more quickly to changes in pollution than geometric means. The 90^{th} percentile was estimated as detailed in NSSP, 1999.

5.3.5. Data treatment and Statistical analysis

Analysis of variance in the monthly MPN values of total coliforms, faecal coliforms and *E. coli* of the six selected sampling points in the mussel beds showed no significant difference between the sampling points. Therefore, the monthly MPN values of the sampling points were pooled together for the mussel beds and arithmetic mean and standard deviation were calculated using the $\log_{10}$ transformed values. Seasonal (pre-monsoon, monsoon and post monsoon) variations of coliform counts in each mussel bed was analysed by one-way ANOVA. Station-wise analysis also was carried out within each season using one-way ANOVA. In each mussel bed the interdependence of the coliform counts of seawater, sediment and mussel tissue was analysed by estimating the Pearson's correlation coefficients. Bioaccumulation indices were calculated as

the ratio of arithmetic mean of MPN values of total coliforms, faecal coliforms and *E. coli* in mussel tissue to that of the overlaying seawater. The indices were also calculated as ratio of the MPN values in tissue to that in sediments.

5.4. Results

5.4.1. Coliform levels in seawater

The counts of total coliforms, faecal coliforms and *E. coli* of seawater from the mussel beds off Someshwara and Surathkal enumerated using MPN method is presented in Table 5.1.

Someshwara: The mean $\log_{10}$ MPN counts of coliforms in seawater from the mussel beds off Someshwara were 2.65±1.09 for total coliforms, 2.60±1.19 for faecal coliforms and 2.07±0.88 for *E. coli*/ 100 ml. The total coliform numbers in seawater was highest in June (3.20 MPN/ 100 ml) decreased from October and reached the lowest value of 0.94 MPN/ 100 ml in December (Fig. 5.1). The faecal coliform count followed similar trend with lowest value of 0.51 MPN/ 100 ml in December and highest in June (3.20 MPN/ 100 ml). Similarly highest *E. coli* count was recorded in June (2.94 MPN/ 100 ml) and lowest in April (0.40 MPN/ 100 ml). Season-wise analysis indicated that the coliform count was very high during monsoon compared to pre-monsoon and post-monsoon (Fig. 5.2). The mean $\log_{10}$ MPN count of coliforms in seawater during monsoon was 3.16±0.29 for total coliforms, 3.11±0.57 for faecal coliforms and 2.60±0.83 for *E. coli*/ 100 ml. The analysis of variance revealed significant seasonal differences in total coliform, faecal coliform and *E. coli* levels in seawater during the study period (Table 5.2).

Surathkal: The MPN counts of coliform bacteria of seawater of Surathkal mussel bed presented less variability compared to Someshwara. The $\log_{10}$ MPN/ 100 ml were 2.58±0.96 for total coliforms; 2.17±0.86 for faecal coliforms and 1.88±0.81 for *E. coli.* Total coliforms ranged from 0.85 MPN/ 100 ml in February to 3.13 MPN/ 100 ml in August (Fig. 5.1). Faecal coliforms ranged from 0.59 MPN/ 100 ml in December to 2.85 MPN/ 100 ml in June and *E. coli* from 0.05 MPN/ 100 ml in December to 2.65 MPN/ 100 ml in June. Similar to the seasonal trends observed in Someshwara beds, higher coliforms levels were noticed in monsoon season than in pre-monsoon and post-monsoon seasons (Fig. 5.2) at Surathkal. The mean $\log_{10}$ MPN counts/100 ml in seawater was 3.00±0.65 for total coliforms, 2.57±0.87 for faecal coliforms and 2.30±0.81 for *E. coli* in monsoon. Analysis of seasonal variability indicated significant difference in coliform counts between seasons (Table 5.2).

Analysis of variance in coliform counts between the mussel beds showed significant differences ($p<0.05$) in total coliform and faecal coliform counts during monsoon and post-monsoon seasons (Table 5.3). During pre-monsoon season no significant difference was observed in coliform counts between the mussel beds.

5.4.2. *Coliform levels in sediment*

Someshwara: The counts of total coliforms, faecal coliforms and *E. coli* in the sediment (log_{10} MPN) of mussel beds are presented in Table 5.4. In sediment samples, the total coliform levels ranged between 1.56 MPN/ 100 g in November and 4.20 MPN/ 100 g in October with a mean log_{10} value of 3.18±1.18 MPN/ 100 g. Faecal coliforms and *E. coli* were absent in sediments during November-December and the highest counts were observed in February and July (Fig. 5.3). The mean log_{10} count of faecal coliforms was 2.34±1.13 MPN/ 100 g and that of *E. coli* was 2.19±1.08 MPN/ 100 g in the sediment samples. Season-wise analysis of coliform counts indicated highest values of total coliforms in sediment during post-monsoon season (3.43±1.46 MPN/ 100 g) followed by monsoon and pre-monsoon seasons (Fig. 5.4). The counts of faecal coliforms and *E. coli* did not follow similar seasonal variations and the highest values were observed in monsoon. The faecal coliform and *E. coli* counts were 2.62±0.52 MPN/ 100 g and 2.30±0.46 MPN/ 100 g respectively during monsoon. Analysis of variance revealed significant difference ($p<0.05$) in coliforms levels in sediment samples between the seasons at Someshwara (Table 5.5).

Surathkal: The numbers of total coliforms, faecal coliforms and *E. coli* in the sediment of Surathkal mussel beds, estimated using MPN technique as log_{10} values are presented in Table 5.4. The coliform counts of Surathkal presented less variability compared to that of Someshwara with mean values of 2.44±0.71 MPN/ 100 g for total coliforms, 2.24±0.96 MPN/ 100 g for faecal coliforms and 1.80±0.86 MPN/ 100 g for *E. coli*. The total coliforms in the sediment samples ranged between 1.26 MPN/ 100 g in October and 3.15 MPN/ 100 g in January. Faecal coliforms count ranged from none in October and December to 2.73 MPN/ 100 g in January. *E. coli* counts during the year ranged from none during October-December months to 2.73 MPN/ 100 g in January. Statistical analyses showed that there is significant difference in the coliform counts in sediments between the seasons (Table 5.5). The total coliforms were higher (2.73±1.13 MPN/ 100 g) during post-monsoon season followed by pre-monsoon and monsoon seasons (Fig. 5.4). The faecal coliforms (2.43±1.34 MPN/ 100 g) and *E. coli* (1.99±1.02 MPN/ 100 g) showed similar trends with higher counts in post-monsoon season.

Analysis of variance in coliform counts between the mussel beds showed significant variation ($p<0.05$) during monsoon season whereas, no significant variation was observed during the pre-monsoon and post-monsoon seasons (Table 5.6).

5.4.3. *Coliform levels in mussel tissue*

Someshwara: The log_{10} MPN of coliforms in tissue samples of mussels from Someshwara mussel beds are presented in Table 5.7. The total coliforms in the tissue samples varied between 2.0 MPN/ 100 g (November) and 4.20 MPN/ 100

g (August) with a mean of 3.37±1.04 MPN/ 100 g. Faecal coliforms presented highest monthly variability, registering counts between 1.44 MPN/ 100 g (February) and 4.04 MPN/ 100 g (August) with a mean of 3.11±1.13 MPN/ 100 g (Fig. 5.5). Count of *E. coli* in the tissue samples ranged between 0.78 MPN/ 100 g (January) and 2.51 MPN/ 100 g (August) with a mean of 2.02±0.98 MPN/ 100 g. Wide seasonal variations were observed in the presence of coliforms in the mussel tissue (Fig. 5.6). Monsoon season registered highest coliform levels with mean $\log_{10}$ value of 3.83±0.78 MPN/ 100 g for total coliforms, 3.68±0.80 MPN/ 100 g for faecal coliforms and 2.40±0.24 MPN/ 100 g for *E. coli*. Variations in the total coliform, faecal coliform and *E. coli* counts in mussel tissue were significant ($p<0.05$) between the seasons (Table 5.8).

Surathkal: At Surathkal mussel beds the coliform counts in mussel tissue were more or less comparable with that of Someshwara beds except for faecal coliforms (Table 5.7). The mean $\log_{10}$ MPN counts were 3.31±1.04 $\log_{10}$ MPN/ 100 g for total coliforms; 2.52±0.90 MPN/ 100 g for faecal coliforms and 2.02±0.95 MPN/ 100 g for *E. coli*. The levels of total coliforms, faecal coliforms and *E. coli* varied from 1.76 MPN/ 100 g (February) to 4.06 MPN/ 100 g (June), 1.58 MPN/ 100 g (February) to 3.22 MPN/ 100 g (August) and 1.33 MPN/ 100 g (December) to 2.41 MPN/ 100g (July) respectively. The MPN values of total coliforms, faecal coliforms and *E. coli* in tissue samples were significantly higher ($p<0.05$) during the monsoon season (Fig. 5.6), when compared with pre-monsoon and post-monsoon seasons (Table 5.8). The mean MPN values during monsoon season were 3.63±0.67 MPN/ 100 g, 2.92±0.83 MPN/ 100 g and 2.29±0.82 MPN/ 100 g respectively for total coliforms, faecal coliforms and *E. coli*.

Coliform counts between the beds within the seasons showed significant differences ($p<0.05$) during monsoon and post-monsoon seasons (Table 5.9).

5.4.4. Bioaccumulation indices

The bioaccumulation indices of coliforms in mussel tissue estimated as a function of their counts in seawater and sediments of mussel beds are presented in Table 5.10.

Someshwara: Generally mussel tissue was found having higher load of coliform bacteria than that of overlaying water in mussel beds, as indicated by bioaccumulation index above unity in most of the months. The bioaccumulation index of total coliforms ranged between 0.4 and 36 at Someshwara. In March, April and October the ratio was above 30 indicating a higher bacterial load in tissue in comparison with the overlaying seawater. The indices for faecal coliforms and *E. coli* ranged between 0.2 and 75.5 and 0.2 and 52.9 respectively with highest values in April. The ratio of coliform bacteria in mussel tissues to the coliform load of sediments varied considerably. The bioaccumulation index calculated for the total coliforms ranged between 0.2 and 68.6. For faecal

coliforms and *E. coli* the indices ranged between zero and 47 and zero and 7.3 respectively. Highest tissue to sediment ratio for total coliforms and faecal coliforms was recorded in August whereas, the highest value for *E. coli* was observed in September.

Surathkal: The bioaccumulation indices of Surathkal indicated wide variations in the range of values compared to that of Someshwara (Table 5.10). The bioaccumulation index for total coliforms, faecal coliforms and *E. coli* was 5.4, 2.3 and 1.4 for mussel tissue to seawater respectively. Highest values of all the three indices were observed in May, one month later compared to Someshwara. The ratio of total coliform count of mussel tissue to the sediments varied considerably during the year. The bioaccumulation index of faecal coliforms and *E. coli* was 1.9 and 1.7 respectively.

5.4.5. Influence of environmental variables on coliform levels

Correlation matrix presenting the Pearson's correlation coefficients between the total coliform, faecal coliform and *E. coli* counts in seawater, sediments and mussel tissue and environmental variables *viz.*, rainfall, temperature and salinity are presented in Tables 5.11, 5.12 and 5.13.

Someshwara: Correlation analysis revealed that the total coliform, faecal coliform and *E. coli* counts of mussel tissue was directly related ($p<0.05$) to the coliform counts of seawater and sediment in mussel beds off Someshwara. Significant positive correlation was also noticed between the coliform counts in seawater, sediment and mussel tissue with rainfall, whereas, salinity and temperature had significant negative influence on the coliform counts of seawater, sediment and tissue.

Surathkal: The total coliform, faecal coliform and *E. coli* counts of mussel tissue was directly related ($p<0.05$) to the coliform counts of seawater while the coliform counts of the mussel tissue was found to be not influenced by its levels in the sediment. Rainfall was found to have significant positive influence on the coliform counts of seawater and mussel tissue whereas, the coliform counts of the sediment was unrelated to the rainfall. The total coliform, faecal coliform and *E. coli* counts of seawater were found negatively related to the salinity and temperature. The coliform counts of mussel tissue were also found to have similar relationship with salinity and temperature except in *E. coli* counts.

5.4.6. Evaluation of sanitary standards of mussel beds

Conformity of mussel growing waters of Someshwara and Surathkal to the microbial standards set by NSSP (1999) based on the faecal coliform counts is presented in Table 5.15. The faecal coliform count (geometric mean) observed was 23 MPN/ 100 ml at Someshwara waters and 19 MPN/ 100 ml at Surathkal waters. These values exceed the faecal coliform level of 14 MPN/ 100 ml

(geometric mean) in seawater approved for unrestricted shellfish harvest or standards set for "approved area" (NSSP, 1999).

In the next category of classification in which the shellfish require depuration or relaying prior to sale, the limits of faecal coliforms are set below the geometric mean of 88/ 100 ml, with fewer than 10% of samples exceeding 260/ 100 ml for a five-tube MPN (Table 5.14). In Someshwara and Surathkal shellfish waters the mean (geometric mean) faecal coliform counts were < 88/ 100 ml but in 25% water samples from Someshwara and 12% water samples from Surathkal, the counts exceeded the limit of 260 MPN/ 100 ml. The samples that exceeded the MPN levels of 260 per/ 100 ml were drawn mostly during the monsoon months.

Considering the fact that the mussel beds along the coastal waters of Dakshina Kannada coast are subjected to bacterial contamination mainly from increased runoff associated with monsoon, the faecal coliforms in the mussel beds were analysed seasonally and were evaluated for their conformity with the standards.

Seasonal changes in faecal coliform counts and their relevance:

In Someshwara waters the geometric mean for faecal coliforms during pre-monsoon season was 8 MPN/ 100 ml and during post-monsoon season was 5 MPN/ 100 ml. These counts were below the 14 MPN/ 100 ml, but in pre-monsoon the 90th percentile MPN value was higher than 43 MPN/ 100 ml (Table 5.15). In 27% of the pre-monsoon seawater samples and 19% of the post-monsoon samples the faecal coliforms levels were above 14/ 100 ml, the standard set for "approved area".

Similarly the faecal coliforms levels in seawater from Surathkal yielded low values during pre-monsoon (10 MPN/ 100 ml) and post-monsoon (13 MPN/ 100 ml) seasons but 90th percentile MPN value was substantially higher than the limit of 43 MPN (Table 5.15). In Surathkal the faecal coliforms in 35% of the pre-monsoon seawater samples and 50% of the post-monsoon samples were above 14/ 100 ml.

Predictably, Someshwara (875 MPN/ 100 ml) and Surathkal (83 MPN/ 100 ml) waters yielded elevated faecal coliform MPN values during monsoon season with geometric mean and 90th percentile values well above the limits. It was observed that all the samples from Someshwara and 87% of the samples from Surathkal exceeded the limit during the season. At Surathkal the faecal coliform counts were ten fold lower than the geometric mean values of faecal coliforms in Someshwara during monsoon season.

When shellfish growing waters are exposed to limited amounts of pollution the shellfish must be depurated or relayed prior to sale, provided the limits of faecal coliforms in such conditions do not exceed 88/ 100 ml and the estimated 90th percentile do not exceed 260 MPN/ 100 ml or with fewer than 10% of samples exceeding 260 MPN/ 100 ml for a five-tube MPN. In Someshwara waters the

faecal coliform levels in 91% of the pre-monsoon water samples and in 100% (all) of the post-monsoon samples were below 88 MPN/ 100 ml. Similarly, in Surathkal, the faecal coliform levels in 94% of the pre-monsoon water samples and in 100% (all) of the post-monsoon samples were below 88 MPN/ 100 ml. Therefore, geometric mean and 90^{th} percentile MPN results from Someshwara and Surathkal were well below the limits for "restricted area" classification during the pre-monsoon and post-monsoon seasons.

5.4.7. Biochemical Oxygen Demand (BOD)

The mean BOD_5 values of Someshwara and Surathkal mussel beds were found to be below 2 mg/l and it ranged between 0.3 and 4.5 mg/l during the period of study. The spatial and temporal variations in BOD values of the mussel beds are presented in Table 5.16.

Someshwara: The mean BOD values of Someshwara mussel bed in general varied between 0.3 and 1.8 mg/l. An exceptional peak of 4.5 mg/l was noticed in September. High BOD values were observed during monsoon and pre-monsoon seasons at Someshwara. Seasonal trend in BOD level is presented in Fig. 5.7. Analysis of variance showed significant difference in BOD levels between the seasons ($p<0.05$) at Someshwara.

***Surathkal*:** The mean BOD values at Surathkal varied from 0.9 to 3.4 mg/l. Analysis of seasonal trends in BOD levels revealed significant differences ($p<0.05$) between seasons at Surathkal (Table 5.17). BOD levels were high in monsoon and post-monsoon seasons.

Between the mussel beds of Someshwara and Surathkal significant variations (Table 5.18) in BOD levels were observed only during post-monsoon ($p<0.05$) season.

Table 5.1. Monthly variations of total coliform, faecal coliform and *E. coli* counts in seawater from mussel beds off Someshwara and Surathkal.

Mussel Bed	Month	Seawater ($\log_{10}$ MPN/ 100ml)		
		Total coliforms	Faecal coliforms	*E. coli*
Someshwara	Jan	1.61±0.39	1.51±0.84	0.85±0.55
	Feb	2.90±1.66	2.90±1.66	1.86±1.08
	Mar	1.47±0.73	1.47±0.73	1.46±0.85
	Apr	1.13±0.60	0.61±0.44	0.40±0.33
	May	1.54±0.50	0.92±0.23	0.92±0.23
	Jun	3.20±0.00	3.20±0.00	2.94±0.69
	Jul	3.14±0.30	3.06±0.42	2.76±0.92
	Aug	3.14±0.30	3.13±0.42	2.08±0.34
	Sep	3.13±0.42	3.03±0.94	1.13±0.31
	Oct	2.30±0.93	1.91±0.76	1.90±0.83
	Nov	1.29±0.36	0.77±0.50	0.77±0.50
	Dec	0.94±0.30	0.51±0.36	0.43±0.35
	Mean	**2.65±1.09**	**2.60±1.19**	**2.07±0.88**
Surathkal	Jan	2.21±0.61	1.69±0.45	1.69±0.45
	Feb	0.85±0.38	0.75±0.36	0.63±0.41
	Mar	1.59±0.63	0.96±0.52	0.89±0.56
	Apr	2.38±0.87	2.34±0.87	2.01±0.80
	May	0.95±0.23	0.74±0.18	0.70±0.34
	Jun	3.00±0.36	2.85±0.69	2.65±0.64
	Jul	2.82±0.71	2.75±0.67	2.42±0.58
	Aug	3.13±0.67	1.29±0.37	0.98±0.24
	Sep	3.11±0.60	1.29±0.59	0.98±0.45
	Oct	2.95±0.53	2.04±0.49	1.16±0.52
	Nov	2.07±0.68	1.44±0.71	0.86±0.54
	Dec	0.97±0.20	0.59±0.36	0.05±0.25
	Mean	**2.58±0.96**	**2.17±0.86**	**1.88±0.81**

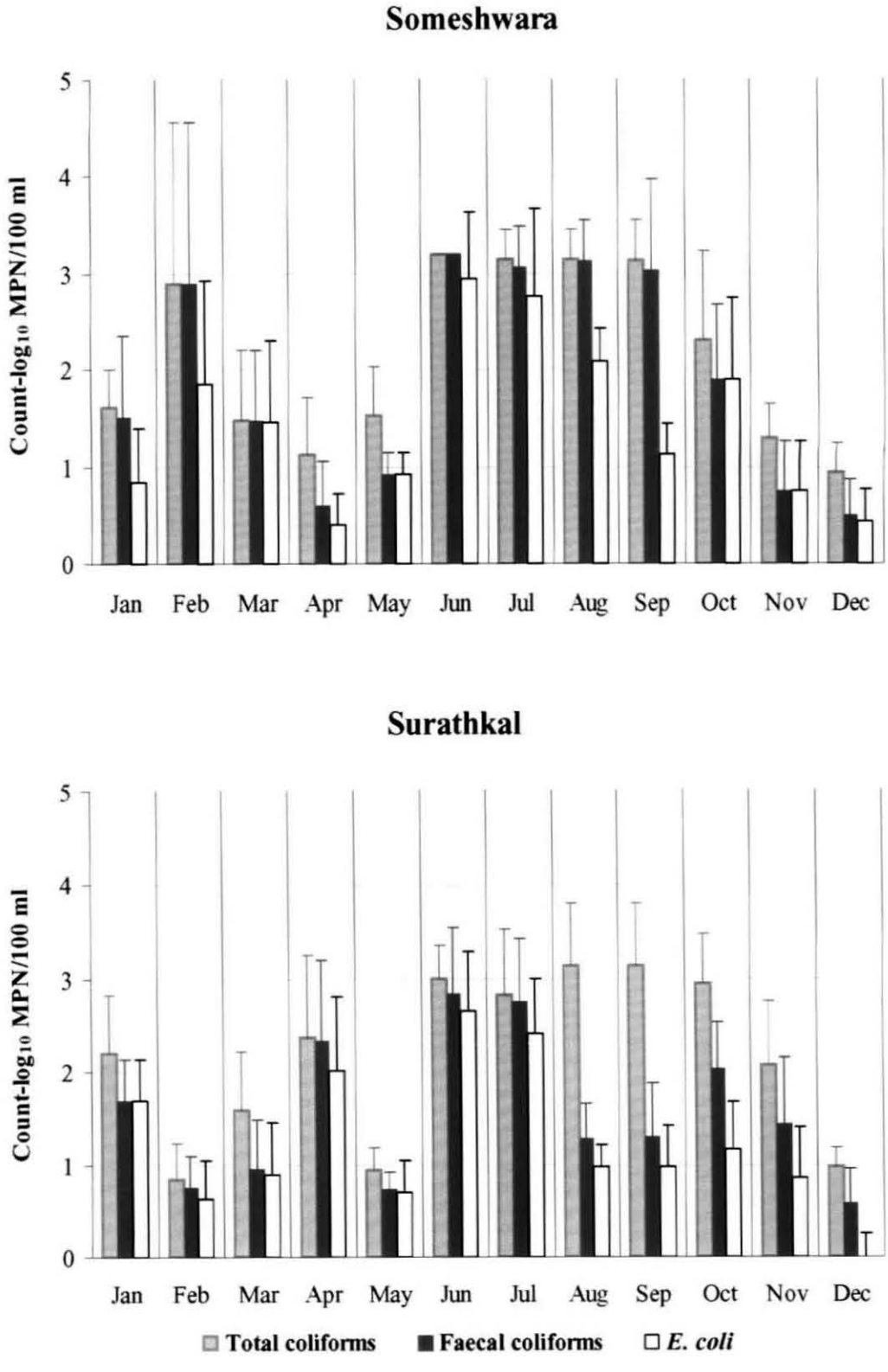

Fig. 5.1. Monthly variations of total coliform, faecal coliform and *E. coli* counts (± SD) in seawater from mussel beds off Someshwara and Surathkal.

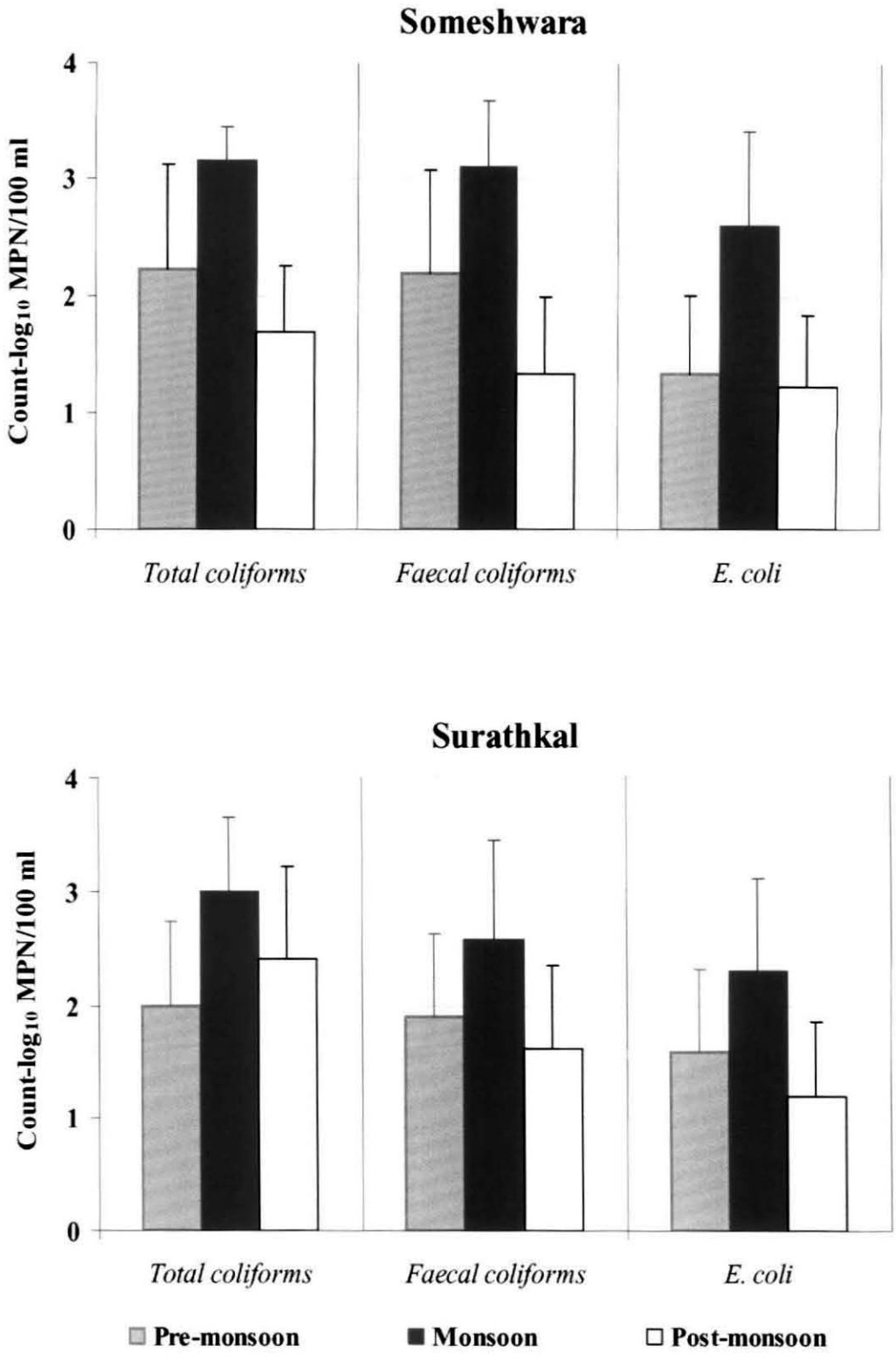

Fig. 5.2. Seasonal variations of total coliform, faecal coliform and *E. coli* counts (± SD) in seawater from mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Table 5.2. Analysis of variance of coliform counts of seawater of the mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Someshwara						
Source		**Sum of squares**	**Degrees of freedom**	**Mean sum of squares**	**F value computed**	***p***
Total coliforms x season	Combined	69.1	2	34.57	**77.5**	*0.000*
	Within groups	40.2	90	0.45		
	Total	109	92			
Faecal coliforms x season	Combined	82.3	2	41.13	**76.3**	*0.000*
	Within groups	48.5	90	0.54		
	Total	131	92			
E. coli x season	Combined	28.5	2	14.26	**29.6**	*0.000*
	Within groups	43.3	90	0.48		
	Total	71.9	92			
Surathkal						
Total coliforms x season	Combined	42.7	2	21.33	**39.1**	*0.000*
	Within groups	59.5	109	0.55		
	Total	102	111			
Faecal coliforms x season	Combined	17.7	2	8.85	**15.1**	*0.000*
	Within groups	63.9	109	0.59		
	Total	81.6	111			
E. coli x season	Combined	15.4	2	7.68	**14.5**	*0.000*
	Within groups	57.8	109	0.53		
	Total	73.2	111			

Table 5.3. Analysis of variance of coliform counts of seawater of the mussel beds (station) off Someshwara and Surathkal within pre-monsoon, monsoon and post-monsoon seasons.

Season	Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Pre-monsoon	Total coliforms x station	Combined	0.3	1	0.28	0.43	*0.514*
		Within groups	54.9	83	0.66		
		Total	55.1	84			
	Faecal coliforms x station	Combined	0.1	1	0.11	0.18	*0.670*
		Within groups	52.3	83	0.63		
		Total	52.4	84			
	E. coli x station	Combined	0.2	1	0.21	0.43	*0.513*
		Within groups	40.4	83	0.49		
		Total	40.6	84			
Monsoon	Total coliforms x station	Combined	1.8	1	1.85	**6.78**	*0.012*
		Within groups	14.2	52	0.27		
		Total	16.0	53			
	Faecal coliforms x station	Combined	14.0	1	14.0	**24.78**	*0.000*
		Within groups	29.3	52	0.56		
		Total	43.3	53			
	E. coli x station	Combined	1.1	1	1.15	1.73	*0.194*
		Within groups	34.5	52	0.66		
		Total	35.7	53			
Post-monsoon	Total coliforms x station	Combined	6.4	1	6.44	**13.46**	*0.000*
		Within groups	30.6	64	0.48		
		Total	37.0	65			
	Faecal coliforms x station	Combined	2.7	1	2.74	**5.70**	*0.020*
		Within groups	30.8	64	0.48		
		Total	33.5	65			
	E. coli x station	Combined	0.3	1	0.27	0.66	*0.418*
		Within groups	26.2	64	0.41		
		Total	26.5	65			

Table 5.4. Monthly variations of total coliform, faecal coliform and *E. coli* counts in sediments from the mussel beds off Someshwara and Surathkal.

Mussel Bed	Month	Sediment (log_{10} MPN/ 100 g)		
		Total coliforms	**Faecal coliforms**	***E. coli***
Someshwara	**Jan**	1.81±0.22	1.81±0.22	1.81±0.22
	Feb	2.83±0.18	2.83±0.18	2.83±0.18
	Mar	1.87±0.00	1.74±0.17	1.26±0.85
	Apr	2.06±1.29	1.26±0.85	1.26±0.85
	May	2.36±0.00	1.56±0.00	1.56±0.00
	Jun	3.38±0.73	2.70±0.60	2.37±0.59
	Jul	2.97±0.00	2.83±0.18	2.46±0.25
	Aug	2.37±0.59	2.37±0.59	2.12±0.44
	Sep	2.40±0.42	2.40±0.42	2.12±0.44
	Oct	4.20±0.00	2.73±0.43	2.73±0.43
	Nov	1.56±0.00	nd	nd
	Dec	1.86±1.04	nd	nd
	Mean	**3.18±1.18**	**2.34±1.13**	**2.19±1.08**
Surathkal	**Jan**	3.15±0.41	2.73±0.43	2.73±0.43
	Feb	1.81±0.12	1.65±0.15	1.53±0.06
	Mar	2.66±0.00	2.66±0.00	1.74±0.16
	Apr	1.52±0.04	1.52±0.04	1.52±0.04
	May	2.33±0.12	1.80±0.22	1.81±0.22
	Jun	2.21±0.22	1.96±0.00	1.56±0.00
	Jul	2.05±0.33	2.05±0.33	1.88±0.22
	Aug	1.92±0.05	1.57±1.02	1.26±0.85
	Sep	1.79±0.27	1.79±0.27	1.66±1.08
	Oct	1.26±0.85	nd	nd
	Nov	2.85±0.65	2.67±1.50	nd
	Dec	1.63±0.90	nd	nd
	Mean	**2.44±0.71**	**2.24±0.96**	**1.80±0.86**

nd: not detected

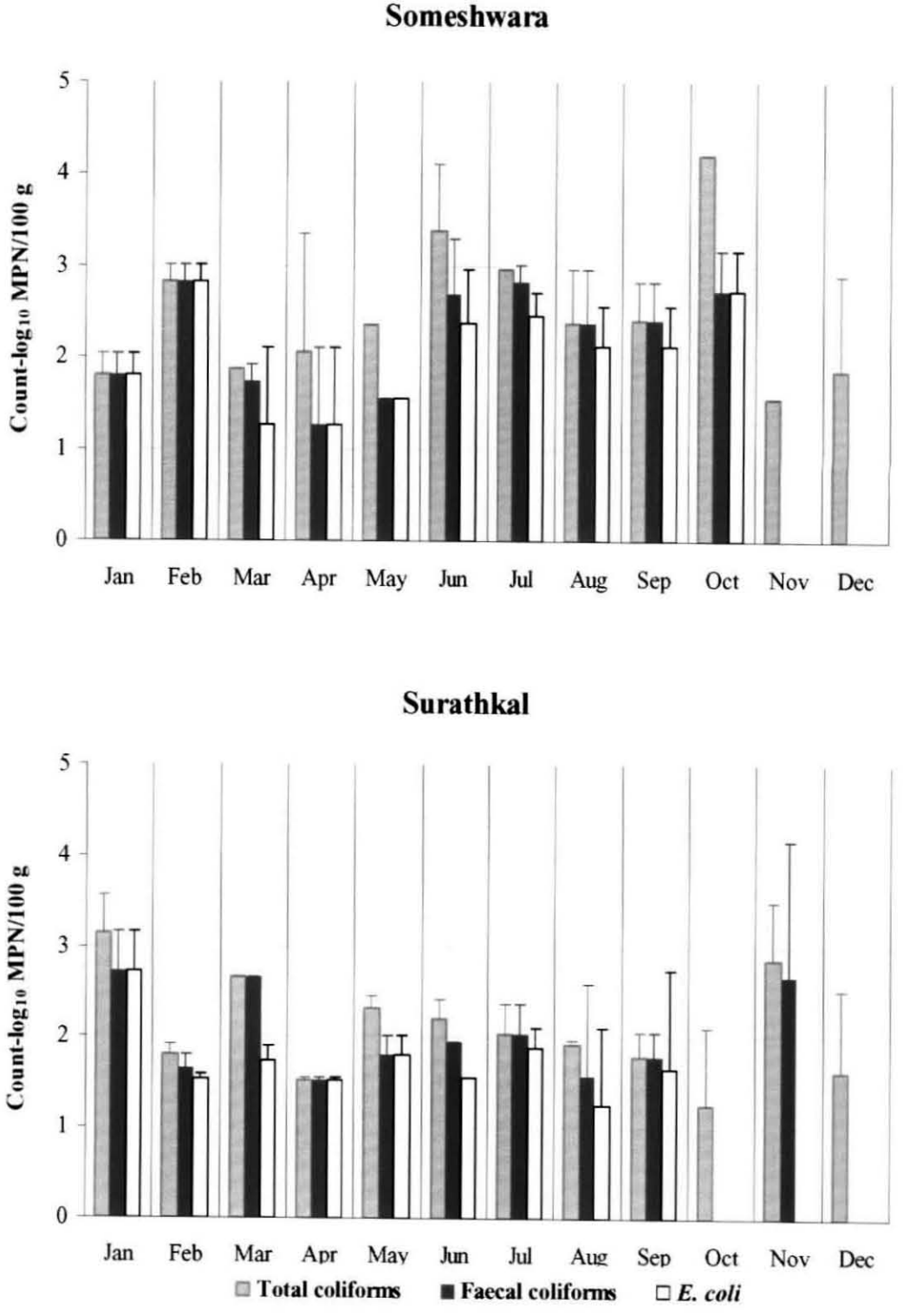

Fig. 5.3. Monthly variations of total coliform, faecal coliform and *E. coli* counts (± SD) in sediments from mussel beds off Someshwara and Surathkal.

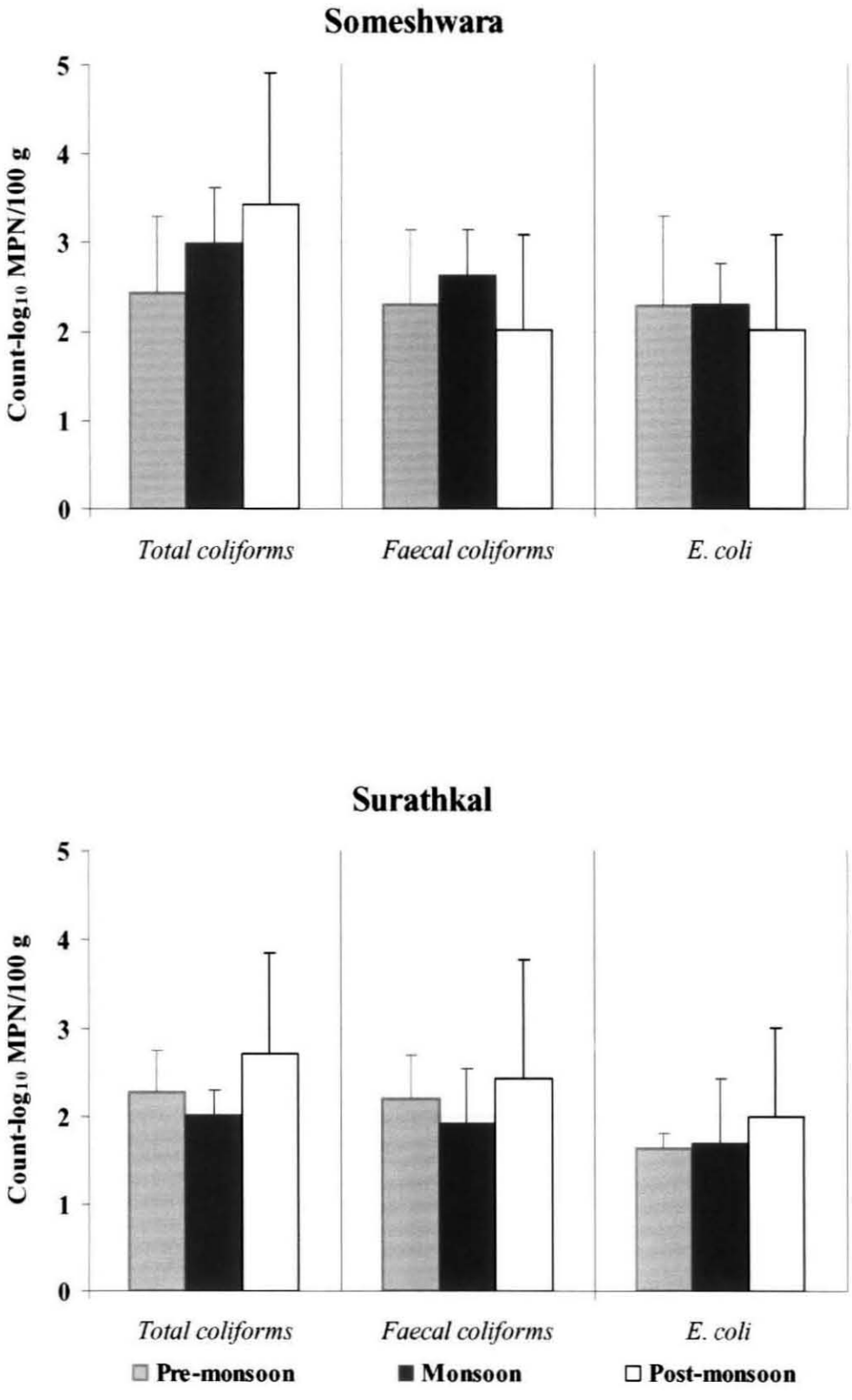

Fig. 5.4. Seasonal variations of total coliform, faecal coliform and *E. coli* counts (± SD) in sediments from mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Table 5.5. Analysis of variance of coliform counts of sediments from the mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Someshwara						
Source		**Sum of squares**	**Degrees of freedom**	**Mean sum of squares**	**F value computed**	***p***
Total coliforms x season	Combined	14.0	2	7.00	**5.6**	*0.005*
	Within groups	101	81	1.25		
	Total	115	83			
Faecal coliforms x season	Combined	41.9	2	20.96	**26.8**	*0.000*
	Within groups	63.4	81	0.78		
	Total	105	83			
E. coli x season	Combined	28.2	2	14.11	**16.5**	*0.000*
	Within groups	69.4	81	0.86		
	Total	97.6	83			
Surathkal						
Total coliforms x season	Combined	0.2	2	0.12	0.2	*0.794*
	Within groups	52.2	102	0.51		
	Total	52.5	104			
Faecal coliforms x season	Combined	16.8	2	8.40	**10.8**	*0.000*
	Within groups	79.1	102	0.78		
	Total	95.9	104			
E. coli x season	Combined	26.0	2	12.99	**26.3**	*0.000*
	Within groups	50.3	102	0.49		
	Total	76.3	104			

Table 5.6. Analysis of variance of coliform counts of sediments of the mussel beds (station) off Someshwara and Surathkal within pre-monsoon, monsoon and post-monsoon seasons.

Season	Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Pre-monsoon	Total coliforms x station	Combined	0.0	1	0.00	0.01	*0.923*
		Within groups	26.5	64	0.41		
		Total	26.5	65			
	Faecal coliforms x station	Combined	0.6	1	0.59	1.42	*0.238*
		Within groups	26.6	64	0.42		
		Total	27.2	65			
	E. coli x station	Combined	0.3	1	0.27	0.69	*0.410*
		Within groups	24.9	64	0.39		
		Total	25.2	65			
Monsoon	Total coliforms x station	Combined	5.5	1	5.47	**24.34**	*0.000*
		Within groups	11.7	52	0.22		
		Total	17.1	53			
	Faecal coliforms x station	Combined	6.4	1	6.37	**18.75**	*0.000*
		Within groups	17.7	52	0.34		
		Total	24.0	53			
	E. coli x station	Combined	6.6	1	6.61	**16.69**	*0.000*
		Within groups	20.6	52	0.40		
		Total	27.2	53			
Post-monsoon	Total coliforms x station	Combined	2.1	1	2.11	1.23	*0.272*
		Within groups	115.4	67	1.72		
		Total	117.6	68			
	Faecal coliforms x station	Combined	1.1	1	1.15	0.78	*0.380*
		Within groups	98.2	67	1.47		
		Total	99.3	68			
	E. coli x station	Combined	1.1	1	1.11	1.01	*0.319*
		Within groups	74.1	67	1.11		
		Total	75.2	68			

Table 5.7. Monthly variations in total coliform, faecal coliform and *E. coli* counts in mussel from mussel beds off Someshwara and Surathkal.

Mussel Bed	Month	Mussel tissue ($\log_{10}$ MPN/ 100 g)		
		Total coliforms	Faecal coliforms	*E. coli*
Someshwara	Jan	2.16±0.47	1.99±0.36	0.78±0.64
	Feb	2.30±0.46	1.44±0.94	1.44±0.94
	Mar	2.98±1.61	1.70±1.09	1.39±0.97
	Apr	2.66±0.42	2.49±0.87	2.12±1.10
	May	2.33±0.17	2.14±0.68	1.80±0.58
	Jun	2.81±0.12	2.50±0.35	2.26±0.21
	Jul	3.05±0.66	2.78±0.53	2.43±0.34
	Aug	4.20±0.00	4.04±0.70	2.51±0.28
	Sep	3.98±0.43	3.86±0.42	2.34±0.08
	Oct	3.85±0.72	1.97±0.28	1.97±0.28
	Nov	2.00±0.45	1.59±0.69	1.44±0.94
	Dec	2.32±1.01	1.71±0.97	1.60±0.94
	Mean	**3.37±1.04**	**3.11±1.13**	**2.02±0.98**
Surathkal	Jan	2.72±0.42	2.37±0.42	2.23±0.33
	Feb	1.76±0.75	1.58±0.68	1.52±0.86
	Mar	2.24±1.20	1.98±1.15	1.96±1.15
	Apr	2.61±0.44	1.99±0.38	1.77±0.62
	May	3.96±0.48	2.72±0.14	2.27±0.28
	Jun	4.06±0.46	3.09±0.40	2.33±0.31
	Jul	3.03±0.43	2.75±0.48	2.41±0.96
	Aug	3.84±0.62	3.22±0.34	2.19±0.24
	Sep	2.89±0.44	2.15±1.19	2.00±1.12
	Oct	3.74±1.11	1.97±0.28	1.97±0.28
	Nov	2.67±0.47	2.46±0.78	1.57±0.85
	Dec	2.76±0.50	2.20±0.29	1.33±0.88
	Mean	**3.31±1.04**	**2.52±0.90**	**2.02±0.95**

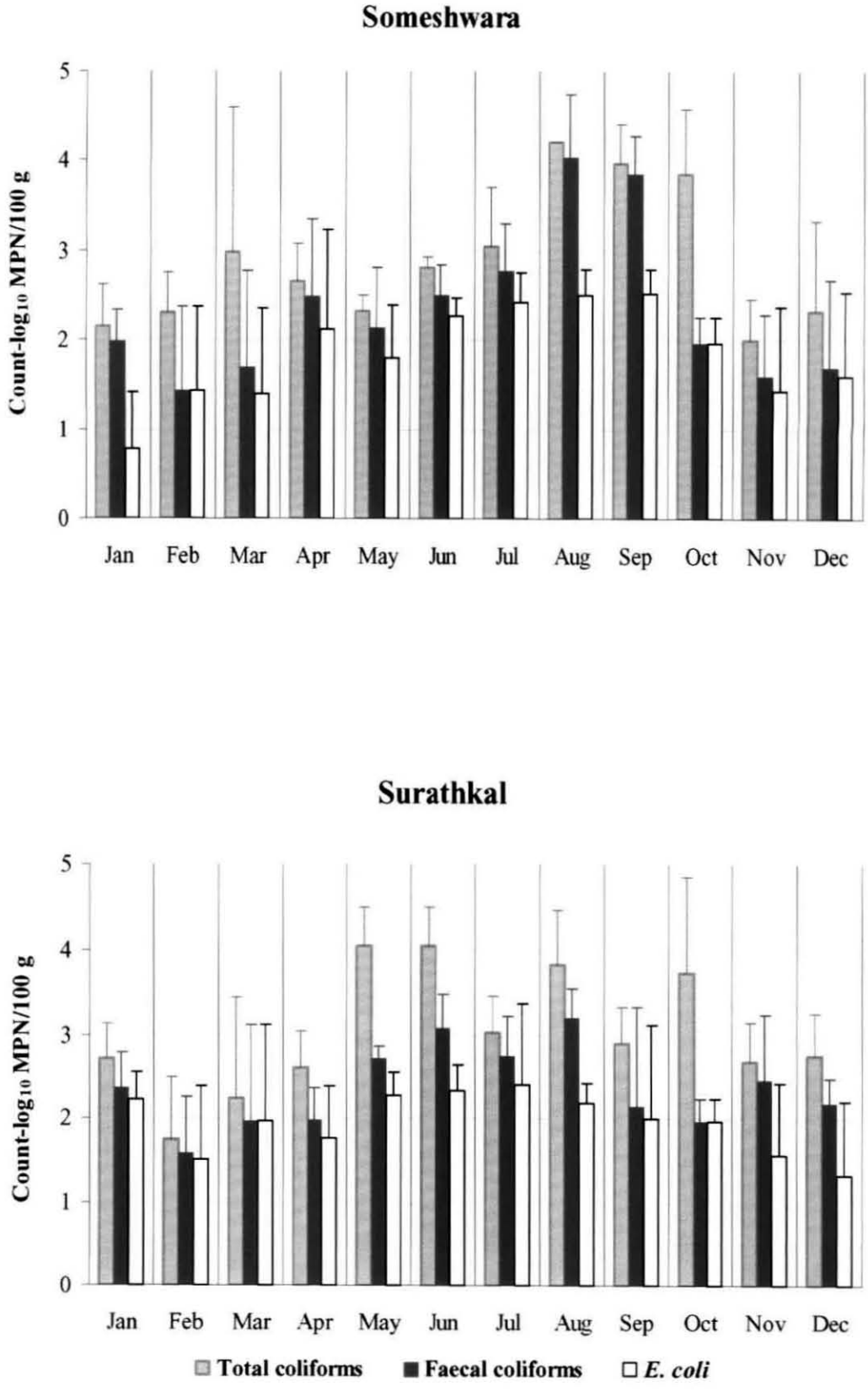

Fig. 5.5. Monthly variations of total coliform, faecal coliform and *E. coli* counts (± SD) in mussel from mussel beds off Someshwara and Surathkal.

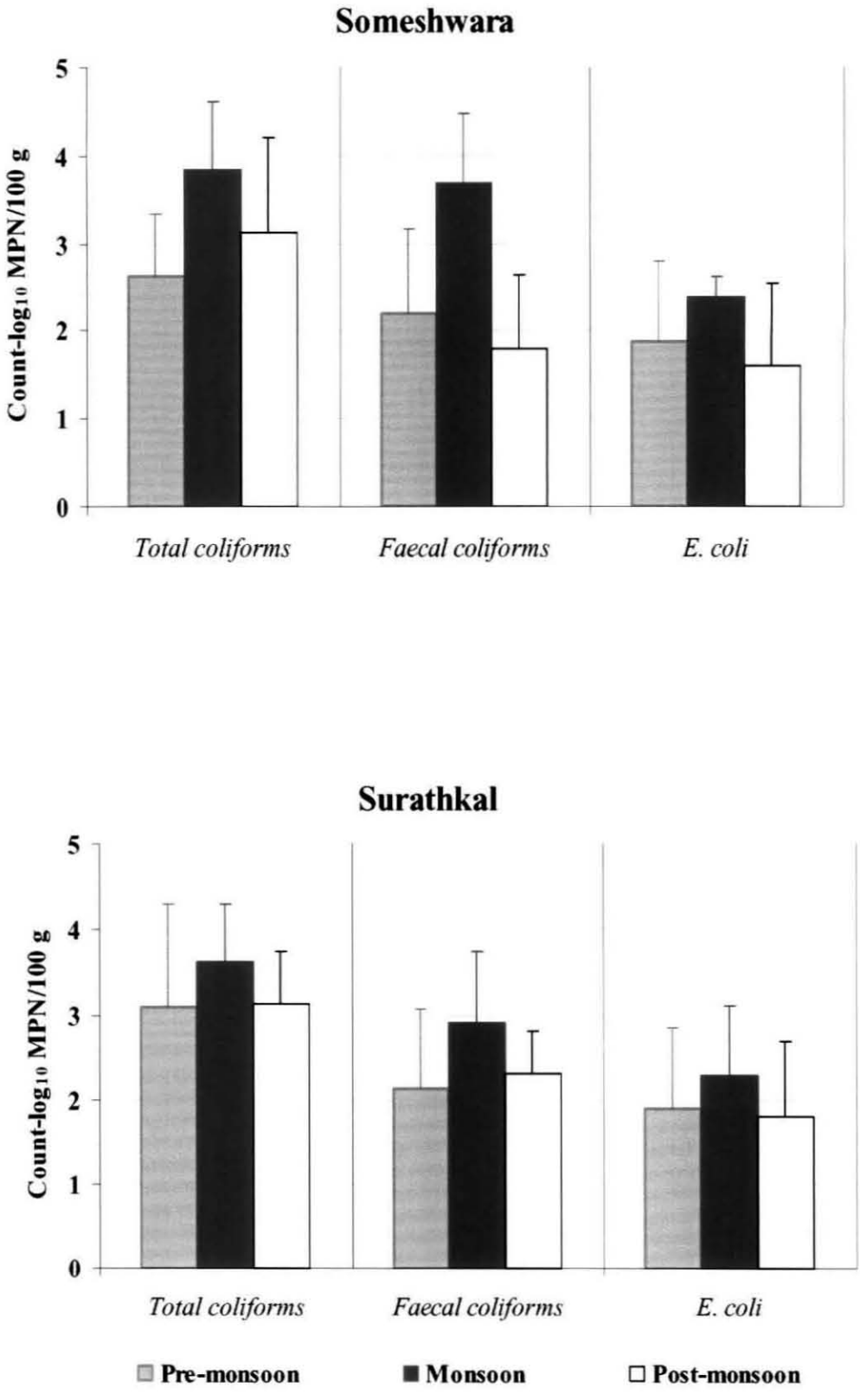

Fig. 5.6. Seasonal variations of total coliform, faecal coliform and *E. coli* counts (± SD) in mussel from mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Table 5.8. Analysis of variance of coliform counts of mussel of the mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Someshwara						
Source		**Sum of squares**	**Degrees of freedom**	**Mean sum of squares**	**F value computed**	***p***
Total coliforms x season	Combined	29.9	2	14.97	**19.1**	*0.000*
	Within groups	72.9	93	0.78		
	Total	103	95			
Faecal coliforms x season	Combined	48.9	2	24.47	**31.6**	*0.000*
	Within groups	71.9	93	0.77		
	Total	121	95			
E. coli x season	Combined	29.2	2	14.60	**21.7**	0.000
	Within groups	62.4	93	0.67		
	Total	91.6	95			
Surathkal						
Total coliforms x season	Combined	29.3	2	14.65	**17.4**	*0.000*
	Within groups	96.0	114	0.84		
	Total	125.3	116			
Faecal coliforms x season	Combined	18.8	2	9.39	**14.3**	*0.000*
	Within groups	74.6	114	0.65		
	Total	93.4	116			
E. coli x season	Combined	10.0	2	4.98	**6.0**	*0.003*
	Within groups	93.9	114	0.82		
	Total	104	116			

Table 5.9. Analysis of variance of coliform counts of mussel of the mussel beds (station) off Someshwara and Surathkal within pre-monsoon, monsoon and post-monsoon seasons.

Season	Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Pre-monsoon	Total coliforms x station	Combined	2.5	1	2.47	2.38	*0.127*
		Within groups	88.3	85	1.04		
		Total	90.7	86			
	Faecal coliforms x station	Combined	0.1	1	0.15	0.16	*0.690*
		Within groups	77.3	85	0.91		
		Total	77.4	86			
	E. coli x station	Combined	0.0	1	0.04	0.04	*0.836*
		Within groups	76.6	85	0.90		
		Total	76.7	86			
Monsoon	Total coliforms x station	Combined	0.5	1	0.52	1.02	*0.318*
		Within groups	26.7	52	0.51		
		Total	27.3	53			
	Faecal coliforms x station	Combined	5.1	1	5.06	**7.54**	*0.008*
		Within groups	34.9	52	0.67		
		Total	40.0	53			
	E. coli x station	Combined	2.0	1	1.96	**4.90**	*0.031*
		Within groups	20.8	52	0.40		
		Total	22.8	53			
Post-monsoon	Total coliforms x station	Combined	6.4	1	6.39	**8.31**	*0.005*
		Within groups	53.9	70	0.77		
		Total	60.3	71			
	Faecal coliforms x station	Combined	9.2	1	9.21	**18.75**	*0.000*
		Within groups	34.4	70	0.49		
		Total	43.6	71			
	E. coli x station	Combined	2.1	1	2.08	2.47	*0.121*
		Within groups	58.9	70	0.84		
		Total	61.0	71			

Table 5.10. Monthly variations in bioaccumulation index of coliforms in mussel tissues

Month	Tissue/seawater			Tissue/sediment		
	Total coliforms	Faecal coliforms	*E. coli*	Total coliforms	Faecal coliforms	*E. coli*
Someshwara						
Jan	3.5	2.9	0.9	2.2	1.5	0.1
Feb	0.2	0.0	0.4	0.3	0.0	0.0
Mar	32.3	1.7	0.8	13.0	0.9	1.4
Apr	34.3	75.5	52.9	4.0	17.1	7.4
May	6.2	16.5	7.6	0.9	3.8	1.8
Jun	0.4	0.2	0.2	0.3	0.6	0.8
Jul	0.8	0.5	0.5	1.2	0.9	0.9
Aug	11.6	8.2	2.7	68.7	47.5	2.4
Sep	7.0	6.7	16.3	37.8	28.6	1.7
Oct	35.9	1.1	1.2	0.4	0.2	0.2
Nov	5.2	6.7	4.7	2.8	0.0	0.0
Dec	23.9	15.8	14.6	2.9	0.0	0.0
Mean	**5.3**	**3.2**	**0.9**	**1.6**	**5.9**	**0.7**
Surathkal						
Jan	3.3	4.8	3.4	0.4	0.4	0.3
Feb	8.1	6.8	7.8	0.9	0.8	1.0
Mar	4.4	10.4	11.8	0.4	0.2	1.7
Apr	1.7	0.5	0.6	12.4	3.0	1.8
May	102	96.1	37.6	42.1	8.3	2.9
Jun	11.4	1.7	0.5	70.6	13.4	6.0
Jul	1.6	1.0	1.0	9.7	5.1	3.3
Aug	5.2	85.3	16.2	83.3	44.5	8.6
Sep	0.6	7.1	10.6	12.9	2.3	2.2
Oct	6.1	0.8	6.5	302	0.0	0.0
Nov	4.0	10.5	5.1	0.7	0.6	0.0
Dec	61.6	40.5	24.5	13.5	0.0	0.0
Mean	**5.4**	**2.3**	**1.4**	**7.5**	**1.9**	**1.7**

Table 5.11. Correlation coefficient (Pearson's) between total coliform (TC) levels in seawater, sediment and mussel tissue from the Someshwara and Surathkal mussel beds.

	Water TC	Sediment TC	Tissue TC	Rain	Salinity	Temperature
Someshwara						
Water TC	1	.398(**)	.455(**)	.695(**)	-.648(**)	-.608(**)
Sediment TC	.398(**)	1	.455(**)	.408(**)	-.331(**)	-0.057
Tissue TC	.455(**)	.455(**)	1	.349(**)	-.227(*)	-.291(**)
Surathkal						
Water TC	1	-0.087	.346(**)	.546(**)	-.562(**)	-.535(**)
Sediment TC	-0.087	1	-.222(*)	0.009	0.091	0.077
Tissue TC	.346(**)	-.222(*)	1	.456(**)	-.327(**)	-.379(**)

Table 5.12. Correlation coefficient (Pearson's) between faecal coliform (FC) levels in seawater, sediment and mussel tissue from the Someshwara and Surathkal mussel beds.

	Water FC	Sediment FC	Tissue FC	Rain	Salinity	Temperature
Someshwara						
Water FC	1	.611(**)	.507(**)	.715(**)	-.662(**)	-.558(**)
Sediment FC	.611(**)	1	.387(**)	.475(**)	-.418(**)	-0.182
Tissue FC	.507(**)	.387(**)	1	.412(**)	-.328(**)	-.388(**)
Surathkal						
Water FC	1	0.118	.231(*)	.576(**)	-.398(**)	-.303(**)
Sediment FC	0.118	1	-0.163	0.089	-0.01	.204(*)
Tissue FC	.231(*)	-0.163	1	.434(**)	-.335(**)	-.400(**)

Table 5.13. Correlation coefficient (Pearson's) between *E. coli* levels in seawater, sediment and mussel tissue from the Someshwara and Surathkal mussel beds.

	Water *E. coli*	Sediment *E. coli*	Tissue *E. coli*	Rain	Salinity	Temperature
Someshwara						
Water *E. coli*	1	.444(**)	.435(**)	.716(**)	-.649(**)	-.366(**)
Sediment *E. coli*	.444(**)	1	.407(**)	.423(**)	-.356(**)	-0.21
Tissue *E. coli*	.435(**)	.407(**)	1	.468(**)	-.407(**)	-.292(**)
Surathkal						
Water *E. coli*	1	.380(**)	.354(**)	.562(**)	-.355(**)	-.215(*)
Sediment *E. coli*	.380(**)	1	0.1	0.146	-0.072	.219(*)
Tissue *E. coli*	.354(**)	0.1	1	.347(**)	-.261(**)	-0.16

**** Correlation is significant at the 0.01 level.**

*** Correlation is significant at the 0.05 level.**

Table 5.14. Classification of shellfish growing areas: Based on microbial examination of seawater samples (NSSP, 1999).

US FDA Classification	Geometric mean	<10% of the sample	Shellfish treatment required	Criteria
Approved	MPN <14/100 ml	MPN <43/100 ml	None	Acceptable water quality; No significant pollution sources.
Conditionally approved	Open Depending on whether conditions of the "approved area" are met.			Example: Rainfall, Wastewater treatment plant functioning.
Restricted	MPN <88/100 ml	MPN <260/100 ml	Depuration or relaying	Evidence of marginal pollution
Conditionally restricted	Open Depending on whether conditions of the "restricted area" are met.			Example: Rainfall, Wastewater treatment plant functioning.
Prohibited	No harvest allowed			Evidence of gross pollution

Table 5.15. Summary of non-point source faecal coliform (MPN/ 100 ml) analysis of mussel growing waters based on the limits set by NSSP (1999).

Mussel bed	Season	Faecal coliforms Geometric mean	90th Percentile	N	% >14 MPN/ 100 ml	% >260 MPN/ 100 ml
Someshwara	Pre-monsoon	8	113	33	27	9
	Monsoon	**875**	**4638**	24	100	83
	Post-monsoon	5	36	36	19	0
	Annual	23	**759**	93	43	25
	Non-monsoon	6	64	69	23	9
Surathkal	Pre-monsoon	10	80	52	35	6
	Monsoon	**83**	**1077**	30	87	33
	Post-monsoon	13	112	30	50	0
	Annual	19	232	112	53	12
	Non-monsoon	11	90	82	40	6

Table 5.16. Monthly variations of BOD levels (± SD) in seawater from the Someshwara and Surathkal mussel beds.

Months	Someshwara (mg/l)	Surathkal (mg/l)
Jan	0.8±0.6	1.3±0.3
Feb	0.7±0.7	1.4±0.4
Mar	1.6±1.0	2.0±1.1
Apr	1.8±1.2	0.9±0.5
May	1.3±1.1	1.4±0.7
Jun	0.3±0.2	2.2±0.9
Jul	0.7±0.3	3.4±2.5
Aug	1.8±0.1	1.6±0.4
Sep	4.5±0.2	1.7±0.3
Oct	0.8±0.6	2.5±0.2
Nov	0.7±0.5	1.7±0.6
Dec	0.7±0.7	1.5±0.5
Mean	1.2±1.1	1.8±1.2

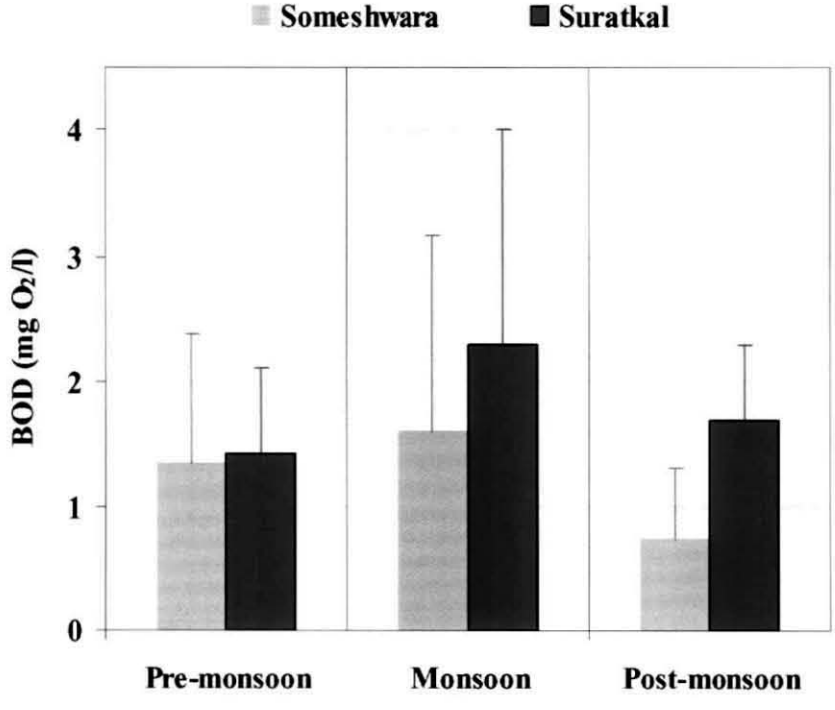

Fig. 5.7. Seasonal variations of BOD levels (± SD) in seawater during pre-monsoon, monsoon and post-monsoon seasons from the Someshwara and Surathkal mussel beds.

Table 5.17. Analysis of variance of Biochemical Oxygen Demand (BOD) levels of seawater from mussel beds off Someshwara and Surathkal during pre-monsoon, monsoon and post-monsoon seasons.

Mussel bed	Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Someshwara	BOD x Season	Combined	14	2	6.8	**5.64**	*0.005*
		Within Groups	137	113	1.2		
		Total	150	115			
Surathkal	BOD x Season	Combined	15	2	7.4	**5.81**	*0.004*
		Within Groups	129	101	1.3		
		Total	144	103			

Table 5.18. Analysis of variance of Biochemical Oxygen Demand (BOD) levels of seawater of the mussel beds (station) off Someshwara and Surathkal within pre-monsoon, monsoon and post-monsoon seasons.

Season	Source		Sum of squares	Degrees of freedom	Mean sum of squares	F value computed	*p*
Pre-monsoon	BOD x station	Combined	0.2	1	0.2	0.19	*0.667*
		Within Groups	71.5	86	0.8		
		Total	71.6	87			
Monsoon	BOD x station	Combined	8.2	1	8.2	3.06	*0.085*
		Within Groups	171.8	64	2.7		
		Total	180.0	65			
Post-monsoon	BOD x station	Combined	15.8	1	15.8	**44.74**	*0.000*
		Within Groups	22.6	64	0.4		
		Total	38.4	65			

5.5. Discussion

Results of the present study demonstrated the presence of faecal coliform bacteria in seawater, sediment and mussel tissue samples frequently in both Someshwara and Surathkal mussel beds indicating that the mussel beds along the coast are subjected to sewage pollution. The mean coliform count in seawater, mussel tissue and sediment generally remained higher at Someshwara compared to Surathkal. In general, elevated levels of total coliforms, faecal coliforms and *E. coli* were noted in mussel tissue and seawater during the monsoon season indicating organic loading of coastal waters during the season. The seasonal variations in coliform counts were more pronounced at Someshwara than Surathkal.

In natural aquatic environment the die-off rates of faecal coliform bacteria entering the system are influenced by various environmental parameters like temperature, salinity, pH, turbidity, solar radiation and predation (NCDEHNR, 1994). The number of bacteria is rapidly reduced in the sea as a result of physical dilution and microbial inactivation, which depend on various environmental factors (Cornax *et al.*, 1990). In shellfish waters as well as in bivalve tissue the survival of microbes is reported to vary with environmental factors, like temperature and salinity and thereby on a seasonal and spatial scale (Hernroth *et al.*, 2002). Apart from these seasonal factors affecting the survival rates of microbes in their natural environment, substantial share in total microbial load of coastal waters is contributed by runoffs.

Seasonal analysis indicated that the counts of total coliforms, faecal coliforms and *E. coli* in seawater, mussel tissue and sediments were highest during monsoon season except in the sediments of Surathkal mussel beds. In several studies, rainfall has been found, to be negatively influencing the coastal bacterial water quality by increasing the levels of pathogens through rainfall associated surface run-off (Raveendran *et al.*, 1990; Howell *et al.* 1995; Lipp *et al.* 2001b; Burton and Pitt 2002 and Ackerman and Weisberg, 2003). Rainfall-associated outbreaks of shellfish vectored diseases have been reported by many studies highlighting the importance of rainfall-associated contamination (Morse *et al.*, 1986 and Bird and Kraa, 1995). The faecal coliform contamination noticed in the present study has probably resulted by the sewage pollution from non-point sources brought in by increased land-runoffs into the coastal waters during rainy season. Raveendran *et al.* (1990) have observed higher counts of enteric bacteria in seawater along the southwest coast of India during monsoon season. Additionally, heavy rainfall would wash sewage contaminated soil into the nearby Nethravati River which in turn empties into the sea. Sunil *et al.* (2004) observed higher levels of total coliform, faecal coliform and *E. coli* in riverine and estuarine areas of Nethravati River and their adjacent coastal waters during monsoon. The water quality of the coastal areas where the river drains into and the mussel beds located in such areas are negatively affected by these inputs

derived from land use and human activities in the surrounding region from non-point sources (Lipp *et al.*, 2001a). In the present study, the influence of rainfall on the coliform levels in mussel tissue and seawater was evident from their significant positive correlation with rainfall.

During monsoon season, the coastal waters are subjected to changes such as decrease in salinity and temperature and increase in turbidity. The results of the present study indicated a sharp increase in coliform counts with the decrease in salinity. Coliform counts of seawater, mussel tissue and sediments were inversely correlated with salinity in Someshwara, where, besides the pathogen load brought in, increase in river run-off temporarily decreases the salinity in river-mouth and thereby influencing the die-off rate of faecal coliforms. In Surathkal, similar trends were observed in the coliform counts of seawater and mussel tissue with salinity except in sediments. Several studies have documented higher concentrations of faecal coliforms and enteric pathogens in areas of low salinity (greater freshwater influence) (Pettibone *et al.*, 1987 and Raveendran *et al.*, 1990). Similarly, Brock *et al.* (1985) while investigating the relationships of rainfall, river flow and salinity with faecal coliform levels in mussel beds noted a positive link between freshwater input and faecal coliform counts. It was observed that the die-off rates of faecal coliforms in seawater and sediment were higher than in freshwater (Anderson *et al.*, 2005).

The survival of faecal coliform and other enteric bacteria in natural waters are reported to vary with variations in temperature and light intensity (Chamberlin and Michell, 1978). Sunlight has been indicated as one of the most important inactivating factors on survival of *E. coli* in estuarine waters (Chandran and Hatha, 2003). In the mussel beds, water temperature was observed to have significant negative relationship with coliform counts in seawater and mussel tissue. The variations in these factors in turn are strongly correlated with rainfall resulting from increased river flow and cooler water temperatures (Lipp *et al.*, 2001b).

During rainy season, increased sediment transport and turbidity also influence the microbial counts of coastal waters by increasing the survival of coliforms (Ferguson *et al.*, 1996 and Payment *et al.*, 2000). Increase in suspended solids and turbidity are reported to contribute to the better survival of faecal coliform bacteria by providing an organic substrate as well as protection from light in addition to a mechanism for transport downstream (Pommepuy *et al.*, 1992). In coastal waters of Someshwara and Surathkal, a relatively high Biological Oxygen Demand (BOD_5) values were observed during monsoon which corresponded with the high levels of suspended particulate matter (SPM) and primary production. Some of the earlier studies have attributed higher BOD values in coastal waters of Dakshina Kannada coast to sewage discharge (Gupta and Bhattacharya, 2003).

During monsoon season the levels of total coliforms, faecal coliforms and *E. coli* in sediment samples showed significant differences between the mussel beds. The counts were considerably higher at Someshwara during heavy rainfall and runoffs as compared to Surathkal. Therefore, coliform levels in sediments at Someshwara showed significant positive correlation with the rainfall. In Surathkal mussel beds, though positive correlations were observed between coliform levels in seawater and tissue with rainfall, no substantial increase in coliform counts of sediments was noticed with the onset of rainfall. Faecal coliform concentrations are influenced by such factors as drainage area characteristics including land uses, faecal pollution sources and runoff potential of different surfaces and landscapes (Burton and Pitt, 2002 and Pitt, 2000). More broadly, numerous other studies have identified river inflows associated with rainfall as well as surface water runoffs as major sources of coastal microbial pollution (Macfarlane, 1996; Eisele *et al.*, 2001; de Abbott *et al.* 2000; Dwight *et al.* 2002; Ackerman and Weisberg, 2003).

The observed bed-wise differences in the relationship between rainfall and coliform counts may be partly attributed to the release of sediment bound bacteria into the water column of the sewage polluted areas due to the disturbance during rainfall and heavy water influx at Someshwara. In estuarine waters, it is reported that the faecal coliforms that settle down in sediment encounter a favourable non-starvation environment (Davies *et al.*, 1995). These bacteria can be circulated back into the water column through weather periods or other turbulence generating processes leading to increased coliform levels in water. This cycle may greatly prolong the influence of a contamination event.

Other factors contributing to high levels of faecal coliforms in coastal waters are the high degree of urbanization and boating activity. Faecal coliform levels in water bodies are directly related to the density of housing, population, animal density and developmental activities (Young and Thackston, 1999). A larger population producing more faecal waste increases the potential for untreated waste to reach water bodies possibly through leaks in sewer lines, pump stations and spills or overflows of untreated sewage. Relatively lower counts of coliforms in sediments at Surathkal suggest reduced sewage pollution with increasing distance from the river mouth due to dispersion, settling and mortality of the bacteria.

The effluents from sewage treatment plants of Mangalore city are released to the Nethravati and Gurpur Rivers, which join together forming the Nethravati-Gurpur estuary near Someshwara (Gupta and Bhattacharya, 2003). The Under Ground Drainage system of the City (Sewerage) collects wastewater from households for primary treatment in the Sewage Treatment Plants (STP). The hitherto adopted primary treatment procedure involves separation of sludge from sewage and then the treated wastewater is released back into the water source. Existing networks have only two STPs, one at Jeppina Mogaru, at the south of the Mangalore city covering an area of 1.88 km^2 (population - 20,704; census

2001) and the other at Kavoor, located at north of old Mangalore city covering an area of 18.24 km^2 (population-1,37,315: census 2001). The effluent from Jeppina Mogaru plant is disposed into the Nethravati River and from Kavoor plant is let into the Gurpur River through a drain. The solid waste collected is dried and disposed off. Since coliforms can remain viable in the sludge as well as in surface soil, the solid wastes disposed could also pose problems during periods of surface run offs. Based on the bacteriological quality of water and sediment samples analysed, Sunil *et al.* (2004) reported that most of the sampling sites of River Nethravati were contaminated with faecal matter from live stock and other animal sources, whereas, in estuarine sites only human faecal contamination was recorded, mainly during monsoon period.

The movement of animal wastes into surface and ground waters is often cited as a major factor contributing to the pollution of available water in many regions, which depends on the land area used for grazing livestock. None of the potential sources are benign and the cumulative loadings can be immense. For example, dog faeces, have been estimated to contain 23 million faecal coliform bacteria per gram (Schueler, 2000) and animal wastes have been identified as key pollution sources in many shellfish contamination studies (Kelsey *et al.*, 2004; Mallin *et al.*, 2001; van Dolah *et al.*, 2000; Weiskel *et al.*, 1996 and White *et al.*, 2000). Consequently, the evaluation of the impact of animal-grazing operations on water quality is an important component in assessment and implementation plans for abatement of pollution from non-point sources. Differentiating animal sources from human sources may not directly change the quality of shellfish harvest area or its classification, but it may play a significant role in the development of environmental management options to reduce bacterial inputs into a shellfish growing area.

In the present study significant positive correlations were observed between the total coliforms, faecal coliforms and *E. coli* counts of the seawater with that of mussel tissue in Someshwara and Surathkal shellfish waters. Similarly, significant positive correlations were observed between coliform levels of sediment and tissue in Someshwara, while no correlation could be established between coliform counts of sediment and tissue in Surathkal beds. In Surathkal, the total coliform and faecal coliform counts of sediment were not related to the counts in overlying seawater. This indicates that the sediments in mussel beds located in the proximity of river discharges were probably contaminated with domestic as well as other organic wastes allowing longer bacterial survival (Gerba, 1996). Therefore, the positive correlation observed between coliforms in sediments and mussel samples are expected at Someshwara. On the contrary, the presence of enteric bacteria in the tissue even in the absence of faecal coliforms in the sediment at Surathkal suggests that, by virtue of their ability to concentrate bacteria, mussels can become actively contaminated from seawater even at considerable distance from the pollution source.

Evaluation of bacterial counts of mussel tissue with reference to the counts of seawater and sediments of the mussel beds indicated that the counts in the mussel tissues are higher than the surrounding environment. Bioaccumulation study is only intended as a general guide; the underlying principle of the accumulation factor approach is that when the uptake of microorganisms is not controlled by the organism's metabolism, their concentration in the organism or accumulation will be proportional to the concentrations in the water or sediment. The coliform bacteria were concentrated by the mussels in varying degrees. The levels of faecal coliforms in the mussels averaged at 3.2 (Someshwara) to 2.3 (Surathkal) times greater than levels in the water to which they are exposed. However, the *E. coli* levels averaged at only 0.9 (Someshwara) and 1.4 (Surathkal) times the levels to which they were exposed in seawater. The ratio of coliform bacteria in mussel tissue in relation to the coliform load of sediments varied considerably. The average bioaccumulation index was 5.9 for faecal coliforms and 0.7 for *E. coli* at Someshwara and 1.9 and 1.7 respectively for faecal coliforms and *E. coli* at Surathkal.

Bioaccumulation was observed high during the March-May and August-October periods. These periods are characterised by the transition from cooler December-February months to warmer summer months and monsoon to post-monsoon months. Warmer temperature increases the filtration rate of the mussels and hence there is a higher probability of bioaccumulation of faecal coliforms from surrounding waters. However, water temperature alone may not explain accumulation rates since the absolute coliform counts in mussel tissue and seawater was found negatively correlated with temperature mainly due to monsoon effects. Further, a higher ratio during the warmer months could possibly be attributed to the higher rate of reduction in coliform counts in seawater with rising temperature compared to the rate of reduction in mussel tissue. This is also possible when shellfish accumulates coliforms present in the shellfish waters and then maintain these levels long after the water have been cleared of the microbes. Additionally, the increase in activity of mussel, such as high filtration rates, enhances the uptake of microbes together with particulate organic matter. Many workers have pointed out the significance of the presence of particles to which microbes can adhere (Pommepuy *et al.*, 1992 and Davies *et al.*, 1995) thereby greatly enhancing its uptake (Hoff and Becker, 1968). Accumulation of microbial species by shellfish and its elimination kinetics is therefore varying with the environmental condition and the season (Burkhardt *et al.*, 1992).

It is interesting to note that the occurrence of elevated levels of faecal coliforms during monsoon months corresponded with the non-fishing periods in the region. With the onset of monsoon, mussel harvests are stopped due to the high turbidity associated with rough weather conditions prevailing in the region. Therefore, though there is a potential health hazard associated with the consumption of mussels without depuration or proper cooking during the season, the probability

of such incidence is minimized due to non-availability of mussels to the consumers.

In mussel growing regions, where mussels are not harvested round the year, in order to determine the compliance with the appropriate water quality standards in terms of faecal coliform counts, the sampling can be restricted to the mussel harvesting seasons or periods when the area is available for harvest (NSSP, 1999). Accordingly, for the overall evaluation of the mussel beds off Dakshina Kannada district, sampling can be limited to the mussel harvesting seasons, as there is definite non-fishing season because of rough weather conditions. Though the geometric mean and 90^{th} percentile of the faecal coliform MPN counts of seawater in Someshwara and Surathkal beds exceeded the limits of 88 MPN/ 100 ml during monsoon season, it was observed that during the pre-monsoon and post-monsoon seasons the faecal coliform counts were below the limits. Since faecal contamination monitoring needs only to address the harvest seasons of the year, the higher levels in monsoon are of less significance. Consequently, the MPN values are indicative of only marginal pollution during the fishing seasons in pre-monsoon and post-monsoon months and hence the mussel beds off Someshwara and Surathkal meet the standards for "restricted area" classification. The area can be therefore described as "seasonally classified" with the period when, the classification defined applies.

During the fishing season, the geometric mean of faecal coliform counts from the mussel growing waters were well below the NSSP limits set for the above classification suggesting that no specific non-point source of faecal coliform is excessively affecting the area. This indicates that during fair weather conditions there is no serious contamination of the mussel beds. However, the area may be closely monitored and the harvest should be permitted under close supervision with management plans calling for temporary closure following rainfall so that the 88/260 standard is met for all combinations of adverse pollution conditions (rainfall, increased river-inflow).

Compliance to the NSSP standards requires depuration of mussels as the faecal coliform counts in the shellfish waters remained above the limits stipulated by FDA for direct consumption. Therefore, the mussel harvested from the area should be subjected to an effective purification process such as relaying or depuration. In many countries as a general practice the mussels are depurated before being sold alive (Jackson and Ogburn, 1999). Generally, incidence of bacterial diseases through consumption of contaminated shellfish can be avoided through depuration and/or adequate cooking or marinating of shellfish (van den Broek *et al.*, 1979). Earlier studies have shown that the load of faecal indicator bacteria in shellfish was eliminated or significantly reduced in period of 24 h by relaying in cleaner area (al-Jebouri and Trollope, 1984). In bivalves the counts of total bacteria, faecal coliforms and bacterial pathogens were reduced by more than 90% after 4 days of depuration (El-Shenawy, 2004).

Evaluation of the sanitary quality of Someshwara and Surathkal mussel beds of Dakshina Kannada District has highlighted the extent of sewage contamination of the coastal waters of the area. Based on the international standards (US FDA NSSP) it is recommended to limit the mussel harvests from Someshwara and Surathkal beds to post-monsoon and pre-monsoon seasons. Someshwara and Surathkal mussel beds during this period can be classified under the "restricted area" category, where it is recommended that the harvested mussels may be relayed or depurated prior to sale.

The present scenario invites attention to the status of organic pollution of the coastal waters of the region and necessitates implementation of regulatory measures to prevent further degradation of the water quality. The outflow from the sewage treatment facilities of the local bodies has high sewage pollution potential and is not monitored regularly. The impact of some of these sources can be reduced, though not eliminated through improved management plans. Until now, water pollution control policies have been concentrated on abatement of municipal and industrial point sources, while the relative contribution from non-point sources is assumed to be negligible. The validity of this assumption in developed countries is questionable since recent studies have shown that one-third of the pollutants entering water bodies come from non-point sources. Therefore, water quality monitoring programmes require the evaluation of non-point source pollutants as well and the implementation of plans to abate such pollutant discharge into the water body. Actions that prevent and control faecal pollution in coastal areas where shellfish are grown and harvested are vital for safeguarding public health and environmental quality.

Establishment and maintenance of proper sewage treatment systems, upland disposal of wastewater treatment plant effluents, eco-friendly land use planning and creation of awareness among coastal communities to minimize non-point sources of contamination are important to protect coastal waters from excessive pollution and to support continued use of the renewable shellfish resources. In the present situations it is highly imperative to establish a shellfish sanitation programme and continuous monitoring of the sanitary standards of the mussels to protect the consumers from shellfish mediated disease outbreaks and to ensure the market of the produce. Continuous monitoring of the variations in sanitary quality of mussel beds in association with the meteorological and hydrographic data will enable developing a forecasting or predictive system which can be used, as followed in the management plans of a number of shellfish growing areas in other parts of the world (Brock *et al.*, 1985)

This study indicates that there is a relationship between the rainfall events and the resulting bacterial contamination of mussel beds and support the concept of restricting the harvests after heavy rainfall. Thus based on the observations of the present study, besides, close monitoring of the level of microbial contaminants and implementation of appropriate coastal management measures

to safeguard shellfish waters from pollution it is advisable to depurate or relay the harvested mussels at least for a period of 24 hours prior to sale.

Summary

Summary

- The thesis presents an account on the physiological response of green mussel, *Perna viridis* (Linnaeus) to the temporal and spatial variations in the environmental parameters of the mussel beds. Condition index, a non-specific biomarker which integrates physiological responses of mussels to multiple stressors is evaluated as the intrinsic response to the variable environment. The cytological response in the mussels is also elucidated with reference to the trace metal and organochlorine concentrations of the shellfish waters. Baseline information on the sanitary quality of the coastal waters and the extent of faecal contamination of the mussel beds is also presented.

- The study area covers the mussel beds off Someshwara and Surathkal of Dakshina Kannada District, Karnataka, where green mussel, *P. viridis* contributes to a significant fishery of commercial importance.

- The variations in water temperature, salinity, dissolved oxygen (DO), pH and the qualitative and quantitative variations in seston of the mussel beds were studied to evaluate their temporal and site-specific differences between the two mussel beds.

- With the onset of monsoon a sharp decline in the water temperature, salinity and pH of the mussel beds was noticed. Bottom water temperature at Someshwara mussel beds varied between 25.20°C in August and 31.15°C in April. At Surathkal mussel beds, water temperature ranged between 26.10°C in August and 31.45°C in May. The mean water temperature of the mussel beds varied significantly ($p<0.05$)

on a seasonal scale with Surathkal waters recording relatively higher values.

- Salinity of the mussel beds showed marked variations on a seasonal scale with low saline conditions prevailing during June-August (monsoon) which steadily increased from August reaching a maximum salinity of 35 ppt in May. At Someshwara mussel beds, mean bottom salinity recorded was 31.98±5.43 ppt whereas, at Surathkal mussel beds it was 32.04±4.01 ppt.

- At Someshwara the mean DO of bottom waters was 6.16±0.94 mg/l and at Surathkal it was 6.45±0.81 mg/l. Seasonal analysis indicated that the DO values of surface and bottom waters were the highest, close to saturation during monsoon, followed by pre-monsoon and post-monsoon.

- pH of mussel beds was found to be alkaline throughout the study, ranging between 7.65 and 8.29. Mean pH of the bottom waters observed at Someshwara was 7.98±0.19 and at Surathkal was 8.00±0.17.

- Besides the variability in the physico-chemical parameters of the mussel bed, the study focused the qualitative and quantitative variations in seston, which primarily decides the food availability in the mussel beds. Seston variability was measured in terms of phytoplankton (chl-a), suspended particulate matter (SPM) and particulate organic matter (POM) levels in shellfish waters.

- At Someshwara, chl-a concentration ranged from 2.70 mg/m^3 in June to 28.42 mg/m^3 in September with a mean of 6.79±5.85 mg/m^3. While at Surathkal, the mean chl-a value was 6.43±2.51 mg/m^3 and presented less variation as compared to Someshwara. The levels of chl-a observed in the mussel beds were above the benchmark for phytoplankton abundance (2 mg/m^3) used for grading "good growing conditions" in mussel growing waters.

- Wide variations were noticed in the level of POM in the mussel beds of the study area. In Someshwara, it varied between 3.18 mg/l in January and 15.27 mg/l in September with a mean of 6.29±5.05 mg/l. POM levels at Surathkal ranged from 2.72 mg/l in November to 21.13 mg/l in June with a mean of 6.45±4.92 mg/l.

- In Someshwara, SPM varied between 16.14 mg/l in January and 56.38 mg/l in July (mean 30.32±17.03 mg/l). In Surathkal mussel beds the variations in SPM levels ranged between 11.80 mg/l in December and 114.73 mg/l in June with a mean of 30.31±25.43 mg/l.

- The mean PIM concentration in the mussel beds of Someshwara was 24.89 ± 13.59 mg/l and ranged between 12.96 mg/l in January and 42.34

mg/l in July. At Surathkal PIM levels presented wide variations with the lowest value of 8.55 mg/l in December and the highest value of 93.60 mg/l in June. The mean PIM at Surathkal was 23.85±20.81 mg/l.

- Principal Component Analysis (PCA) was used to determine the dominant patterns of change in environmental parameters (temperature, salinity, pH, DO, rainfall, chl-a, POM, SPM, PIM, POM/SPM, chl-a/POM, PIM%) of the mussel beds in order to explain the importance of each element in the overall variability. The analysis classified the twelve environmental factors into four clusters or groups.

- In mussel beds off Someshwara, the most significant group forming the first principal component included the physico-chemical parameters like salinity, temperature, pH, DO and rainfall (43.75% of the variance). Factors forming the second principal component at Someshwara were the quality of seston, represented by the organic content of the seston together with the percentage of inorganic suspended particles, associated with the variations in river run-off and precipitation.

- At Surathkal, the magnitude of variations in physico-chemical parameters was secondary (PC2; 21.2% variance) when compared to Someshwara. The most significant elements forming the first principal component at Surathkal was seasonal variations in seston (SPM, PIM and POM) quantity demonstrated by 37.3% of the variance and high positive loading in SPM.

- Condition index (CI), expressed as the ratio of soft tissue weight to the shell weight, is one of the indices, generally regarded as an indicator of the health status of mussels. At Someshwara CI ranged from 56.43 in July to 124.23 in March with a mean value of 94.06±36.31. At Surathkal it ranged from 41.00 in June to 186.59 in March with a mean value of 123.21±49.82. The CI was significantly different between stations and seasons ($p<0.05$).

- Meat yield or percentage edibility also displayed similar patterns as CI among the two mussel beds. The mean percentage edibility was 21.91±5.34 and 26.50±7.05 at Someshwara and Surathkal respectively, with the highest in September. Meat yield or percentage edibility also displayed similar patterns as CI among the two mussel beds.

- The study indicated that there is obvious seasonal variation in the mussel condition with a general trend towards lower values in the monsoon. Besides the seasonal variability, site-specific differences among the mussel beds investigated were also observed. Higher condition indices were recorded in mussels collected at Surathkal, whereas, the condition index recorded at Someshwara was always lower than Surathkal regardless of the sampling season

- The variations in CI, with reference to the changes in the environmental variables were analysed by multiple regression for evaluating the influence of individual parameters as well as their combined effects. Water temperature emerged as the most significant variable explaining 35% of the variation in CI. Seston content (POM and chl-a) of the mussel beds along with water temperature accounted for 56% of the variations in CI. Temperature along with food availability displayed the strongest positive influence on mussel condition.

- Discriminant analysis was performed to discriminate distinctive condition status profiles in mussels having high CI ratio (CI_{high}) and low CI ratio (CI_{low}) upon changes in environmental parameters. The parameters weighed high in the model for the referred differentiation for CI were chl-a and temperature. The CI_{high} exhibited the greatest discriminatory success. Analysis of the monthly CI ratio, mean water temperature and Chl-a levels indicated that 83.9% of the CI_{high} was associated with high chl-a and high water temperature group whereas, 72.4% of CI_{low} were associated with low chl-a and low water temperature regime.

- The concentration of selected trace metals and organochlorine pesticides (OCPs) in the coastal waters of the mussel beds was explored to evaluate the quality of coastal waters. The soft tissue concentration of Cd in green mussels from Someshwara varied from nd (below detectable limits) to 0.57, Pb from nd to 6.72, Cu from nd to 7.06, Zn from 1.99 to 34.7, Ni from nd to 0.9 and Fe from nd to 223.8 ppm wet wt. Similarly, the soft tissue concentrations of Cd in mussels from Suratkal varied from nd to 3.35, Pb from nd to 3.68, Cu from nd to 12.37, Zn from 3.79 to 43.48, Ni from nd to 12.23 and Fe from nd to 46.62 ppm wet wt.

- The mean seawater concentration of ∑OCPs (Aldrin, α-BHC β-BHC, γ-BHC, *o,p'*-DDD, *p,p'*-DDD, *p,p'*-DDE *o,p'*-DDT, *p,p'*-DDT dieldrin, endrin, heptachlor, heptachlor epoxide) at Someshwara was 0.164±0.236 ppb and at Surathkal it was 0.081±0.088 ppb. Endrin was the major OCP detected in seawaters both at Someshwara and Surathkal mussel beds. DDT and its metabolites (∑DDT) contributed 40% to the OCPs of Someshwara, whereas, at Surathkal it formed only 8.8%. α-BHC contributed the major share to ∑BHC fraction in the seawaters of mussel beds forming 13% and 27% of the total OCPs at Someshwara and Surathkal respectively.

- The mean tissue concentration of organochlorine pesticides at Someshwara was 0.431±0.019 ppb wet wt. Similarly, at Surathkal the OCPs concentration was 0.536±0.023 ppb. In mussel tissue, heptachlor epoxide contributed the maximum share of the OCPs, forming 98.2% and 60.8% at Someshwara and Surathkal respectively. Among BHC, only α-BHC was detected in mussel tissue forming 1.8% of the OCPs

concentration at Someshwara. At Surathkal, α-BHC concentration was relatively higher when compared to Someshwara and it contributed 27% to the OCPs concentrations in the mussel tissue.

- The cytological response in mussels to chemical contaminants was studied in terms of lysosomal membrane stability by the Neutral Red Retention Assay (NRRA).

- The Neutral Red Retention Assay (NRRA) results revealed that the lysosomes from the green mussel population in Someshwara had the capacity to retain the dye for an average retention time of 122±11 minutes. Similarly the average retention time in mussels from Surathkal was 127±8 minutes. Analysis of variance in the retention time indicated that there is no significant difference between the two mussel beds.

- The biomarker response (lysosomal membrane stability) of mussels corresponded with the tissue burden of contaminants from the mussel beds of the area. The levels of OCPs and trace metals were very low in the mussel beds with concentrations below detectable limit in many of the tissue samples. Furthermore, the increased retention time indicates the absence of a range of other contaminants as well in the shellfish waters. Less retention time is indicative of a higher degree of general stress due to pollution and retention times <60 min indicate severely impaired health.

- The sanitary quality of the shellfish waters was studied for collecting baseline information on the extent of faecal contamination of mussel beds along the Dakshina Kannada Coast. The mean MPN counts ($\log_{10}$ MPN/100 ml) of coliforms in seawater from the mussel beds off Someshwara were 2.65±1.09 for total coliforms, 2.60±1.19 for faecal coliforms and 2.07±0.88 for *E. coli.* At Surathkal waters the values were 2.58±0.96 for total coliforms; 2.17±0.86 for faecal coliforms and 1.88±0.81 for *E. coli.*

- In the sediments off Someshwara mussel beds, the counts ($\log_{10}$ MPN/100 g) of total coliforms, faecal coliforms and *E. coli* were 3.18±1.18, 2.34±1.13 and 2.19±1.08 respectively. At Surathkal the respective counts were 2.44±0.71 for total coliforms, 2.24±0.96 for faecal coliforms and 1.80±0.86 for *E. coli.*

- The mean MPN values ($\log_{10}$ MPN/100 g) of coliforms in tissue samples of mussels from Someshwara were 3.37±1.04 for total coliforms, 3.11±1.13 for faecal coliforms and 2.02±0.98 for *E. coli.* At Surathkal the coliform levels in mussel tissue were more or less comparable with that of Someshwara except for faecal coliforms. The MPN counts ($\log_{10}$ MPN/ 100 g) were 3.31±1.04 for total coliforms; 2.52±0.90 for faecal coliforms and 2.02±0.95 for *E. coli.*

- The total coliform, faecal coliform and *E. coli* counts of mussel tissue were positively correlated ($p<0.05$) to the coliform counts of seawater in mussel beds off Someshwara and Surathkal.

- Geometric mean and 90th percentile MPN results from Someshwara and Surathkal were well below the limits for "restricted area" classification during the pre-monsoon and post-monsoon seasons, based on the international standards (US FDA, NSSP, 1999).

- The study indicated significant spatial and temporal variations in physico-chemical parameters of the mussel beds off Someshwara and Surathkal. Mussel beds off Someshwara presented wider variations in environmental parameters compared to the mussel beds off Surathkal. The variability in condition index (CI) of green mussels, helped in understanding the response of the mussel population to natural stress factors and evaluation of the overall health of the two mussel beds along the Dakshina Kannada coast. The physico-chemical parameters of mussel bed off Surathkal offer a more conducive environment for the growth of mussels, characterised by better CI with low seasonal variations. Water temperature and the organic content of the seston (POM and chl-a) of mussel beds were the most significant variable influencing the CI. The levels of organochlorine pesticides (OCPs) and trace metals in the shellfish waters were very low, with concentrations below detectable limit in many of the tissue samples. The cytological responses (lysosomal membrane stability) in mussels corresponded with the tissue burden of contaminants from the mussel beds of the area. The results of this study suggest that the current OCPs levels of the Someshwara and Surathkal mussel beds are unlikely to result in any significant environmental impacts in the near future. The study indicates that there is a relationship between the rainfall events and the resulting faecal contamination of the mussel beds off Dakshina Kannada coast and support the concept of restricting the mussel harvests after heavy rainfall.

References

References

Ackerman, D. and S.B. Weisberg, 2003. Relationship between rainfall and beach bacterial concentrations on Santa Monica Bay beaches. *J. Water and Health*, 1(2): 85-89.

Ajithakumar, B.S., 1984. Reproductive physiology of Indian species of the genus *Perna* (fly. Mytilidae). Ph.D Thesis, Cochin Univ. of Sci. and Tech., p.179.

Alagarswami, K. 1980. Review on controlled breeding of bivalves of aquaculture importance In: T. Subramonium and S. Varadarajan (Eds.) *Progress in Invertebrate Reproduction and Aquaculture*, 194-204.

Alagarswami, K. 1991. Production of cultured pearls, Published by the Indian Council of Agricultural Research, New Delhi, p.111.

al-Jebouri, M.M. and D.R. Trollope, 1981. The *Escherichia coli* content of *Mytilus edulis* from analysis of whole tissue or digestive tract. *J. Appl. Bacteriology*, 51: 135-142.

al-Jebouri, M.M. and D.R. Trollope, 1984. Indicator bacteria in freshwater and marine molluscs. *Hydrobiologia,* 111: 93-102.

Anderson, I.C., M. Rhodes and H. Kator, 1979. Sublethal stress in *Escherichia coli*: a function of salinity. *Appl. Environ. Microbiol.*, 38(6): 1147-1152.

Anderson, I.C., M.W. Rhodes and H.I. Kator, 1983. Seasonal variation in survival of *Escherichia coli* exposed *in situ* in membrane diffusion chambers containing filtered and non-filtered estuarine water. *Appl. Environ. Microbiol.*, 45(6): 1877-1883.

Anderson, K.L., J.E. Whitlock and V.J. Harwood, 2005. Persistence and differential survival of faecal indicator bacteria in subtropical waters and sediments. *Appl. Environ. Microbiol.,* 71(6): 3041-3048.

APHA, 1984. Compendium of methods for the microbiological examination of foods. 2nd Edition, American Public Health Association, Washington DC.

APHA, 1995. Standard methods for the examination of water and waste water. 19th Edition, American Public Health Association, Washington DC.

APHA, 1998. Standard methods for the examination of water and waste water. 20th Edition, American Public Health Association, Washington DC.

Appukuttan, K.K., K.S. Mohamed, V. Kripa, P.K. Ashokan, M.K. Anil, G. Sasikumar, T.S. Velayudhan, P. Laxmilatha, K.P.S. Koya, P. Radhakrishnan, M. Joseph, P.S. Alloycious, V.G. Surendranathan, M.P. Sivadasan, D. Nagaraja, J. Sharma and M.S. Naik, 2001. Survey of green mussel seed resources of Kerala and Karnataka. *Mar. Fish. Infor. Serv., T & E Ser.*, 168: 12-19.

Appukuttan, K.K., T.P. Nair and K.T. Thomas, 1989. Spat settlement of brown mussel *Perna indica* (Kuriakose and Nair) in the southwest coast of India. *J. Mar. Biol. Ass. India*, 31(1&2): 266-275.

Appukuttan, K.K., T.P. Nair, M. Joseph and K.T. Thomas, 1980. Culture of brown mussel at Vizhijam. *Coastal aquaculture: Mussel Farming Progress and Prospects, Cent. Mar. Fish. Res. Inst. Bulletin*, 29: 30-32.

Appukuttan, K.K., V. Kripa, T.S. Velayudhan, K.S. Mohamed, A.C.C. Victor, P.S. Kuriakose, P. Laxmilatha and P. Muthiah, 2000. Bivalve Mariculture in India - A Success Story in coastal ecosystem Development. In: V.N. Pillai (Ed.) Published by Asia Pacific

Association of Agricultural Research Institutions, FAO. (APAARI Publication 2000/1), p. 56.

ASQAP (Australian Shellfish Quality Assurance Program), 2004. Operations Manual. p.172. (*available at http://www.pir.sa.gov.au/~sasqap/asqap_manual_final.pdf).*

Auffret, M., S. Rousseau, I. Boutet, A. Tanguy, J. Baron, D. Moraga and M. Duchemin, 2006. A multiparametric approach for monitoring immunotoxic responses in mussels from contaminated sites in Western Mediterranea. *Ecotoxicol. Environ. Saf.*, 63(3): 393-405.

Aunaas, T., J.P. Denstad and K.E. Zachariassen, 1988. Ecophysiological importance of the isolation response of hibernating blue mussels (*Mytilus edulis*). *Mar. Biol.*, 98: 415-419.

Ayres, P.A., 1975. The quantitative bacteriology of some commercial bivalve shellfish entering British markets. *J. Hyg.*, 74: 431-440.

Bacon, G.S., B.A. MacDonald, J.E. Ward, 1998. Physiological responses of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles I. Feeding activity and selection. *J. Exp. Mar. Biol. Ecol.*, 219: 105-125.

Balachandran, V.K., M.S. Rajagopalan and V.K. Pillai, 1989. Chlorophyll *a* and phaeopigment as indices of biological productivity in the inshore surface waters off Cochin. *Indian J. Fish.*, 36(3): 227-237.

Bamber, R.N., 1990. The effects of acidic seawater on three species of lamellibranch mollusc. *J. Exp. Mar. Biol. Ecol.*, 143: 181-191.

Banse, K., 1987. Seasonality of phytoplankton chlorophyll in the central and northern Arabian Sea. *Deep-sea Res.*, 34(5-6): 713-723.

Banse, K., 1988. Estimates of average phytoplankton division rates in the open Arabian Sea. *Indian J. Mar. Sci.*, 17(1): 31-36.

Banse, K., 1996. On the possible causes of the seasonal phytoplankton blooms along the southwest coast of India. *Indian J. Mar. Sci.*, 25: 283-289.

Barille, L., J. Prou, M. Heral and D. Razet, 1997. Effects of high natural seston concentrations on the feeding, selection and absorption of the oyster *Crassostrea gigas* (Thunberg). *J. Exp. Mar. Biol. Ecol.*, 212(2): 149-172.

Bayne, B.L., 1975. Reproduction in bivalve molluscs under environmental stress. In: F.J. Vernberg (Ed.) Physiological ecology of estuarine or estuarine organisms, Univ. of South Carolina Press, Columbia, South Carolina., 259-277.

Bayne, B.L., 1989. Measuring the biological effects of pollution: The mussel watch approach. *Water Sci. Technol.*, 21: 1089-1100.

Bayne, B.L. and C.M. Worall, 1980. Growth and production of mussels *Mytilus edulis* from two populations. *Mar. Ecol. Prog. Ser.*, 3: 317-328.

Bayne, B.L. and R.C. Newell, 1983. Physiological energetics of marine molluscs. In: Wilburg, K.M., Saleuddin, A.S.M. (Eds.) *The Mollusca*, Vol. 4 Academic Press, London, 407-515.

Bayne, B.L. and A.J.S. Hawkins, 1992. Ecological and physiological aspects of herbivory in benthic suspension feeding molluscs. In: John, D.M., Hawkins, A.J.S., Price, J.M. (Eds.), Plant Animal Interactions in the Marine Benthos. Oxford Univ. Press, Toronto, 265- 287.

Bayne, B.L., R.J. Thompson, J. Widdows, 1976. Physiology: I. In: B.L. Bayne, (Ed.) *Marine mussels: Their Ecology and Physiology*. Cambridge Univ. Press, London, 121-293.

Bayne, B.L., A.J.S. Hawkins and E. Navarro, 1987. Feeding and digestion by the mussel *Mytilus edulis* L. (Bivalvia, Mollusca) in mixtures of silt and algal cells at low concentrations. *J. Exp. Mar. Biol. Ecol.*, 111: 1-22.

Bayne, B.L., A.J.S. Hawkins, E. Navarro and I.P. Iglesias, 1989. Effects of seston concentration of feeding, digestion and growth in the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.*, 55: 47-54.

Bayne, B.L., J.I.P. Iglesias, A.J.S. Hawkins, E. Navarro, M. He´ral, J.M. Deslous-Paoli, 1993. Feeding behaviour of the mussel, *Mytilus edulis* L.; responses to variations in both quantity and organic content of seston. *J. Mar. Biol. Assoc. U.K.*, 73: 813-829.

Benakappa, S., M.P.M. Reddy and V. Hariharan, 1980. Hydrographic conditions in the fishing grounds off Mukka-Kaup, South Kanara. *Mahasagar: Bull. Natl. Inst. Oceanogr.*, 13(1): 1-7.

Benson, A.J., D.C. Marelli, M.E. Frischer, J.M. Danforth and J.D. Williams, 2001. Establishment of the green mussel, *Perna viridis* (Linnaeus 1758), (Mollusca: Mytilidae) on the west coast of Florida. *J. Shellfish Res.*, 20: 21-29.

Berg, J.A. and R.I.E. Newell, 1986. Temporal and spatial variation in the composition of seston available to the suspension feeder *Crassostrea virginica*. *Estuar. Coast. Shelf Sci.*, 23: 375-386.

Bernard, F.R., 1989. Uptake and elimination of coliform bacteria by four marine bivalve mollusks. *Can. J. Fish. Aquat. Sci.*, 46: 1592-1599.

Bhaskar, P.V., E. Cardozo, A. Giriyan, A. Garg and N.B. Bhosle, 2000. Sedimentation of particulate organic matter in the Dona Paula Bay, west coast of India during November to May 1995-97. *Estuaries*, 23(2): 722-734.

Bhattacharya, B.K., 1991. Hydrographical characteristics of Gurpur estuary receiving sewage effluents. M.F.Sc. Thesis. Univ. Agrl. Sci., Bangalore, p. 149.

Bhattathiri, P.M.A., A. Pant, S. Sawant, M.U. Gauns, S.G.P. Matondkar, R. Mahanraju, 1996. Phytoplankton production and chlorophyll distribution in the eastern and central Arabian Sea in 1994-1995. *Curr. Sci.*, 71(11): 857-862.

Bird, P. and E. Kraa, 1995. Overview of the 1990 viral gastroenteritis outbreak from oysters. In: Poggi, R., Le Gall, J.Y. (Eds.) Purification des Coquillages (Shellfish Depuration). Proceedings of the Second International Conference on Shellfish Depuration, Rennes, France, 1992. IFREMER - Centre de Brest, Plouzane, 31-36.

Blackmore, G. and W.X. Wang, 2003. Inter-population differences in Cd, Cr, Se and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. *Aquat. Toxicol.*, 62(3): 205-218.

Boggs, S., 1987. Principles of Sedimentology and Stratigraphy. Merrill Publ. Co., London, 784.

Borenfreund, E. and J.A. Puerner, 1985. Toxicity determined in vitro by morphological alterations and neutral red absorption. *Toxicology Letters*, 24(2-3): 119-124.

Bricelj, V.M. and R.E. Malouf, 1984. Influence of algal and suspended sediment concentrations on the feeding physiology of the hard clam *Mercenaria mercenaria*. *Mar. Biol.*, 84:155-165.

Brock, R.L., G.R. Galbraith and B.A. Benseman, 1985. Relationship of rainfall, river flow and salinity to faecal coliform levels in a mussel fishery. *N. Z. J. Mar. Freshw. Res.*, 19: 485-494.

Brown, J.R. and E.B. Hartwick, 1988. Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas* II. Condition index and survival. *Aquaculture*, 70: 253-267.

Brown, R.J., T.S. Galloway, D. Lowe, M.A. Browne, A. Dissanayake, M.B. Jones and M.H. Depledge, 2004. Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. *Aquat. Toxicol.*, 66(3): 267-278.

Burkhardt, W. and K.R. Calci, 2000. Selective Accumulation may account for shellfish-associated viral illness. *Appl. Environ. Microbiol.*, 66(4): 1375-1378.

Burkhardt, W., W.D. Watkins and S.R. Rippey, 1992. Seasonal effects on accumulation of microbial indicator organisms by *Mercenaria mercenaria*. *Appl. Environ. Microbiol.*, 58(3): 826-831.

Burns, K.A., M.G. Ehrhardt, J. MacPherson, J.A. Tierney, G. Kananen and D.Connelly, 1990. Organic and trace metal contaminants in sediments, seawater and organisms from two Bermudan harbours. *J. Exp. Mar. Biol. Ecol.*, 138(1-2): 9-34.

Burton, G.A. and R.E. Pitt, 2002. *Stormwater Effects Handbook: A Toolbox for Watershed Managers Scientists and Engineers*. CRC Press, Inc. Boca Raton, Florida. (*available at http://www.epa.gov/ednnrmrl/ publish/book/hand book/index.htm).*

Cabelli, V.J. and W.P. Heffernan, 1970. Accumulation of *Escherichia coli* by the Northern Quahaug. *Appl. Microbiol.*, 19(2): 239-244.

Cajaraville, M.P., I. Olabarrieta and I. Marigomez, 1996. *In vitro* activities in mussel haemocytes as biomarkers of environmental quality: a case study in the Abra estuary (Biscay Bay). *Ecotoxicol. Environ. Saf.*, 35(3): 253-260.

Camacho, A.P., U. Labarta and R. Beiras, 1995. Growth of mussels (*Mytilus edulis* and *M. galloprovincialis*) on cultivation rafts: influence of seed source, cultivation site and phytoplankton availability. *Aquaculture,* 138: 349-362.

Camus, L., B.E. Grøsvik, J.F. Børseth, M.B. Jones and M.H. Depledge, 2000. Stability of lysosomal and cell membranes in haemocytes of the common mussel (*Mytilus edulis*): effect of low temperatures. *Mar. Environ. Res.*, 50(1-5): 325-329.

Canty, M.N., J.A. Hagger, R.T.B. Moore, L. Cooper and T.S. Galloway, 2007. Sublethal impact of short term exposure to the organophosphate pesticide azamethiphos in the marine mollusc *Mytilus edulis*. *Mar. Pollut. Bull.*, 54(4): 396-402.

Canzonier, W.J., 1971. Accumulation and elimination of Coliphage S-13 by the hard clam, *Mercenaria mercenaria*. *Appl. Microbiol.*, 21(6): 1024-1031.

Castro, M., M.M. Santos, N.M. Monteiro and N. Vieira, 2004. Measuring lysosomal stability as an effective tool for marine coastal environmental monitoring. *Mar. Environ. Res.*, 58(2-5): 741-745.

Ceballos, V.B.P., M.A. Martinez, F.G. Dominguez and M.V. Fuerte, 2000. Reproductive cycle of the rugosse pen shell, *Pinna rugosa* Sobewey, 1835 (Mollusca: Bivalvia) from Bahia Concepcion, Gulf of California and its relation to temperature and photoperiod. *J. Shellfish. Res.*, 19(1): 95-99.

Chamberlin, C.E. and R. Mitchell, 1978. A decay model for enteric bacteria in natural waters, In: R. Mitchell (Ed.) Water Pollution Microbiology, Vol. 2, Wiley Interscience, New York. p. 325-348.

Chan, H.M., 1989. Temporal and spatial fluctuations in trace metal concentrations in transplanted mussels in Hong Kong. *Mar. Pollut. Bull.*, 20(2): 82-86.

Chandran, A. and A.A.M. Hatha, 2003. Survival of *Escherichia coli* in a tropical estuary. *S. Pac. J. Nat. Sci.* 21: 41-46.

Channeshappa, K.T., 1991. Studies on the oceanographic features in relation to plankton and pelagic fisheries in the Arabian Sea off Mangalore region. Ph.D Thesis Univ. Agril. Sci., Bangalore, 243 pp.

Chatterji, A., Z.A. Ansari, B.S. Ingole, A.H. Parulekar, 1984. Growth of the green mussel, *Perna viridis* L., in a seawater circulating system. *Aquaculture,* 40: 47-55.

Chen, M., X. Wanzhu, W. Congming, W. Lifeng, F. Shaohua, L. Pilian, S. Weiying, W. Guitai and H. Jianli, 1998. Study on the technique of collecting semi-artificial spat of mussel *Perna viridis* in Quanzhou Bay and Weitou Bay. *Stud. Mar. Sin.*, 39: 51-57.

Cheng, T.C., 1983a. Triggering of immunologic defense mechanisms of molluscan shellfish by biotic and abiotic challenge and its applications. *Mar. Technol. Soc. J.*, 17: 18-25.

Cheng, T.C., 1983b. Internal defense mechanisms of molluscs against invading microorganisms: Personal reminiscences. *Trans. Amer. Microsc. Soc.*, 102: 185-193.

Cheshuk, B.W., G.J. Purser and R. Quintana, 2003. Integrated open-water mussel (*Mytilus planulatus*) and Atlantic salmon (*Salmo salar*) culture in Tasmania, Australia. *Aquaculture,* 218: 357-378.

Cheung, V.V., R.J. Wedderburn and M.H. Depledge, 1998. Molluscan lysosomal responses as a diagnostic tool for the detection of a pollution gradient in Tolo Harbour, Hong Kong. *Mar. Environ. Res.*, 46(1-5): 237-241.

Chiu, S.T., F.S. Lam, W.L. Tze, C.W. Chau and D.Y. Ye, 2000. Trace metals in mussels from mariculture zones, Hong Kong. *Chemosphere*, 41(1-2): 101-108.

Chong, K. and W.X. Wang, 2000. Bioavailability of sediment-bound Cd, Cr and Zn to the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. *J. Exp. Mar. Biol. Ecol.*, 255(1): 75-92.

Chong, K. and W.X. Wang, 2001. Comparative studies on the biokinetics of Cd, Cr and Zn in the green mussel *Perna viridis* and the Manila clam *Ruditapes philippinarum*. *Environ. Pollut.*, 115(1): 107-121.

Chu, F.L.E. and A.K. Volety, 1997. Disease processes of the parasite *Perkinsus marinus* in the eastern oyster *Crassostrea virginica*: minimum dose for infection initiation and interaction of temperature, salinity and infective cell dose. *Dis. Aquat. Org.*, 28: 61-68.

Chu, K.H., W.M. Cheung and S.K. Lau, 1990. Trace metals in bivalves and sediments from Tolo Harbour, Hong Kong. *Environ. Int.*, 16(1): 31-36.

CIESM, 2002. Mediterranean mussel watch-designing a regional program for detecting radionuclides and trace contaminants. *CIESM workshops series*, 15, 136 pp.

Classie, D., 1989. Chemical contamination of French coasts. *Mar. Pollut. Bull.*, 20: 523-528.

Coles, J.A., S.R. Farley and R.K. Pipe, 1995. Alteration of the immune response of the common marine mussel *Mytilus edulis* resulting from exposure to cadmium. *Dis. Aquat. Org.*, 22: 59-65.

Colley, D.R.J., R.G. Bell and M.D. Andrea, 1994. Sunlight inactivation of enterococci and faecal coliforms in sewage effluent diluted in seawater. *Appl. Environ. Microbiol.,* 60(6): 2049-2058.

Cornax, R., M.A. Morinigo, P. Romero, J. Borrego, 1990. Survival of pathogenic microorganisms in seawater. *Curr. Microbiol.,* 20: 293-298.

Cranford, P.J., C.W. Emerson, B.T. Hargrave and T.G. Milligan, 1998. *In situ* feeding and absorption responses of sea scallops *Placopecten magellanicus* (Gmelin) to storm-induced changes in the quantity and composition of the seston. *J. Exp. Mar. Biol. Ecol.*, 219: 45-70.

Crosby, M.P. and L.D. Gale, 1990. A review and evaluation of bivalve condition index methodologies with a suggested standard method. *J. Shellfish Res.*, 9(1): 233-237.

Cruz, F.M., G.T. Quesada, S.B. Mas, D.N. Lees, K. Henshilwood, A.K. Allard, A.C.C. Hansson, B.E. Hernroth, A. Vantarakis, A. Tsibouxi, M. Papapetropoulou, M.D. Furones and R. Girones, 2002. Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom. *Appl. Environ. Microbiol.*, 68(12): 5990-5998.

Cruz, F.M., A.K. Allard, A.C.C. Hansson, K. Henshilwood, B.E. Hernroth, J. Jofre, D.N. Lees, F. Lucena, M. Papapetropoulou, R.E. Rangdale, A. Tsibouxi, A. Vantarakis and R. Girones, 2003. Evaluation of potential indicators of viral contamination in shellfish and their applicability to diverse geographical areas. *Appl. Environ. Microbiol.*, 69(3): 1556-1563.

Dahlhoff, E.P. and B.A. Menge, 1996. Influence of phytoplankton concentration and wave exposure on the ecophysiology of *Mytilus californianus*. *Mar. Ecol. Prog. Ser.*, 144: 97-107.

Dailianis, S., G.P. Domouhtsidou, E. Raftopoulou, M. Kaloyianni and V.K. Dimitriadis, 2003. Evaluation of neutral red retention assay, micronucleus test, acetylcholinesterase activity and a signal transduction molecule (cAMP) in tissues of *Mytilus galloprovincialis* (L.), in pollution monitoring. *Mar. Environ. Res.*, 56(4): 443-470.

Dame, R.F. and T.C. Prins, 1998. Bivalve carrying capacity in coastal ecosystems. *Aquat. Ecol.*, 31: 409-421.

Davenport, J., 1979. The isolation response of mussels (*Mytilus edulis*) L. exposed to falling seawater concentrations. *J. Mar. Biol. Ass. U.K.*, 59: 124-132.

Davenport, J., 1985. Osmotic control in marine animals. In: M.S. Laverack (Ed.) Physiological adaptations of marine animals, Vol. 39, Symp. Soc. Exp. Biol. The company of Biologists Ltd., University of Cambridge, 207-245.

Davenport, J. and X. Chen, 1987. A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). *J. Molluscan Stud.*, 53: 293-297.

Davies, C.M., J.A.H. Long, M. Donald and N.J. Ashbolt, 1995. Survival of faecal microorganisms in marine and freshwater sediments. *Appl. Environ. Microbiol.*, 61(5): 1888-1896.

Davies, I.M. and J.M. Pirie, 1980. Evaluation of a 'Mussel watch' project for heavy metals in Scottish coastal waters. *Mar. Biol.*, 57: 87-93.

de Abbott, L.S., G.D. Lewis and R.G. Creese, 2000. Temporal and Spatial Distribution of Enterococcus in Sediment, Shellfish Tissue and Water in a New Zealand Harbour. *J. Shellfish Res.*, 19(1): 423-429.

de Jonge, V.N. and J.E.E. van Beusekom, 1992. Contribution of resuspended microphytobenthos to total phytoplankton in the Ems estuary and its possible role for grazers. *Neth. J. Sea. Res.*, 30: 91-105.

de Sousa, S.N., K. Sawkar and P.V.S.S.D. Rao, 1996. Environmental changes associated with monsoon induced upwelling, off central west coast of India. *Indian J. Mar. Sci.*, 25: 115-119.

de Zwaan, A. and M. Mathieu, 1992. Cellular biochemistry and endocrinology. In: Gosling E. (Ed.) The mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier Science, Amsterdam, 223-307.

de Zwaan, A., P. Cortesi, G. van den, T.J. Roos and K.B. Storey, 1991. Differential sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. *Mar. Biol.*, 111: 343-351.

Deepanjali, A., H.S. Kumar, I. Karunasagar and I. Karunasagar, 2005. Seasonal variation in abundance of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters along the southwest coast of India. *Appl. Environ. Microbiol.*, 71(7): 3575-3580.

Demers, S., J.C. Therriault, E. Bourget and A. Bah, 1987. Resuspension in the shallow sublittoral zone of a macrotidal estuarine environment: wind influence. *Limnol. Oceanogr.*, 32: 327-339.

Denis, L., E. Alliot and D. Grzebyk, 1999. Clearance rate responses of Mediterranean mussels, *Mytilus galloprovincialis*, to variations in the flow, water temperature, food quality and quantity. *Aquat. Living Resour.*, 12(4): 279-288.

Devassy, V.P., 1983. Plankton production associated with cold water incursion into the estuarine environment. *Mahasagar: Bull. Natl. Inst. Oceanogr.*, 16(2): 221-233.

Dileep, V., H.S. Kumar, Y. Kumar, I. Karunasagar, M. Nishibuchi and I. Karunasagar, 2003. Application of polymerase chain reaction for the detection of *Vibrio parahaemolyticus* associated with tropical, seafoods and coastal environment. *Lett. Appl. Microbiol.*, 36: 423-427.

Domouhtsidou, G.P., S. Dailianis, M. Kaloyianni and V.K. Dimitriadis, 2004. Lysosomal membrane stability and metallothionein content in *Mytilus galloprovincialis* (L.), as biomarkers Combination with trace metal concentrations. *Mar. Pollut. Bull.*, 48: 572-586.

Dore, W.J., M. Mackie and D.N. Lees, 2003. Levels of male-specific RNA bacteriophage and *Escherichia coli* in molluscan bivalve shellfish from commercial harvesting areas. *Lett. in Appl. Microbiol.*, 36: 92-96.

Dowd, M., 1997. On predicting the growth of cultured bivalves. *Ecol. Modell.*, 104: 113-131.

Dwight, R.H., J.C. Semenza, D.B. Baker and B.H. Olson, 2002. Association of Urban Runoff with Coastal Water Quality in Orange County, California. *Water Environment Research.*, 74(1): 82-90.

EC (European Commission), 1979. Council Directive 79/923/EEC of 30 October 1979 on the quality required of shellfish waters. Official Journal of the European Community, No. L281, 10/11/1979, 47-55.

EC (European Commission), 2001. Commission Regulation (EC) No 466/2001 of 8 March 2001. Official Journal of European Communities 1.77/1.

EC (European Commission), 1991. Council Directive 91/492/EEC of 15 July 1991 laying down the health conditions for the production and the placing on the market of live bivalve mollusks. Official Journal of the European Communities, No. L268, 24/09/1991, 1-14.

Edmonds, R.L., 1976. Survival of coliform bacteria in sewage sludge applied to a forest clearcut and potential movement into groundwater. *Appl. Environ. Microbiol.*, 32(4): 537-546.

Eisele, W., B.J. Zimmer and R. Connell, 2001. Pressures on New Jersey Shellfish Waters. *J. Shellfish Res.*, 20(3): 1301-1304.

El-Shenawy, N.S., 2004. Heavy-metal and microbial depuration of the clam *Ruditapes decussatus* and its effect on bivalve behavior and physiology. *Environ. Toxicol.*, 19(2): 143-153.

Emmett, B., K. Thompson and J.D. Popham, 1987. The reproductive and energy storage cycles of two populations of *Mytilus edulis* from British Columbia. *J. Shellfish Res.*, 6: 29-36.

ENVIS, 2002. Estuaries of India, State-of-the-art report (Ed.) T. Balasubramanian and S.A. Khan Publication Series: 1/2002, *www.casmbenvis.nic.in/down loads/estuaries%20 status.pdf*, 202 pp.

FAO, Fisheries Department, Fishery Information, Data and Statistics Unit. FISHSTAT Plus: Universal software for fishery statistical time series. Ver. 2.3. 2000, *www.fao.org/fi/statist/statist.asp.*

FDA, 1999. Pesticide Analytical Manual, third ed., Vol. 1. Food and Drugs Administration, USA.

FDA, 2001. Fish and Fisheries Products Hazards and Controls Guidance, 3rd Ed. Center for Food Safety and Applied Nutrition, US Food and Drug Administration.

Ferguson, C.M., B.G. Coote, N.J. Ashbolt and I.M. Stevenson, 1996. Relationships between indicators, pathogens and water quality in an estuarine system. *Water Res.*, 30: 2045-2054.

Fernley, P.W., M.N. Moore, D.M. Lowe, P. Donkin and S. Evans, 2000. Impact of the Sea Empress oil spill on lysosomal stability in mussel blood cells. *Mar. Environ. Res.*, 50(1-5): 451-455.

Fisher, H., 1984. Cadmium body burden/shell weight of mussels: a precise index for environmental monitoring. *Comm. Meet Int. Coun. Explor. Sea CM-ICESE,* 41: 1-19.

Flemer, D.A., 1969. Chlorophyll analysis as a method of evaluating the standing crop of phytoplankton and primary production. *Chesapeake Sci.*, 10: 301-306.

Florence, T.M., 1972. Determination of trace metals in marine samples by anodic stripping voltammetry. *J. Electro-analytical Chemistry*, 35: 237-245.

Francioni, E., A.L.R. Wagener, A.L. Scofield, M.H. Depledge and B. Cavalier, 2007. Evaluation of the mussel *Perna perna* as a biomonitor of polycyclic aromatic hydrocarbon (PAH) exposure and effects. *Mar. Pollut. Bull.,* 54(3): 329-338.

Fréchette, M. and E. Bourget, 1985. Energy flow between the pelagic and benthic zones: factors controlling particulate organic matter available to an intertidal mussel bed. *Can. J. Fish. Aquat. Sci.*, 42, 1158-1165.

Frechette, M., E. Bourget, C. Butman and W.R. Geyer, 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis L. Limnol. Oceanogr.*, 34: 19-36.

Fung, C.N., J.C.W. Lam, G.J. Zheng, D.W. Connell, I. Monirith, S. Tanabe, B.J. Richardson and P.K.S. Lam, 2004. Mussel-based monitoring of trace metal and organic contaminants along the east coast of China using *Perna viridis* and *Mytilus edulis. Environ. Pollut.,* 127(2): 203-216.

Gardner, J.P.A., 2002. Effects of seston variability on the clearance rate and absorption efficiency of the mussels *Aulacomya maoriana, Mytilus galloprovincialis* and *Perna canaliculus* from New Zealand. *J. Exp. Mar. Biol. Ecol.,* 268: 83-101.

Garen, P., S. Robert and S. Bougrier, 2004. Comparison of growth of mussel, *Mytilus edulis*, on longline, pole and bottom culture sites in the Pertuis Breton, France. *Aquaculture*, 232: 511-524.

Gaspar, M.B. and C.C. Monteiro, 1999. Gametogenesis and spawning in the subtidal white clam *Spisula solida*, in relation to temperature. *J. Mar. Biol. Ass. UK.*, 79: 753-755.

Gerba, C.P., J.B. Rose, C.N., Haas and K.D. Crabtree, 1996. Waterborne rotavirus: a risk assessment. *Water Res.*, 30: 2929-2940.

Giese* A.C., 1959. Comparative physiology, Annual reproductive cycles of marine invertebrates. *Annu. Rev. Physiol.*, 21: 547-576.

Glynn, D., L.T.B. McHugh, A. Rowe, J. Costello and E. McGovern, 2003. Trace metal and chlorinated hydrocarbon concentration in shellfish from Irish waters, 2000. *Marine*

Environmental and Health Series, 7. Marine Institute, Marine Environment and Food Safety Services Abbotstown, Dublin, 21 pp.

Gopinathan, C.P. R. Gireesh and K.S. Smitha, 2001. Distribution of chlorophyll 'a' and 'b' in the eastern Arabian Sea (west coast of India) in relation to nutrients during post monsoon. *J. Mar. Biol. Ass. India,* 43(1&2): 21-30.

Gore, P.S. and S.Y.S. Singbal, 1973. Ecology and production in two sandy beaches of Goa Part A. Distribution of bacteria in Colva and Siridaon in relation to certain environmental parameters. *Fish. Tech.*, 10(1): 55-60.

Gore, P.S., T.S.G. Iyer, O. Raveendran and P.V. Unnithan, 1980. Isolation and significance of *Salmonella* sp. from some sandy beachs of Kerala. *Mahasagar: Bull. Natl. Inst. Oceanogr.*, 13(2): 147-152.

Gore, P.S., O. Raveendran, T.S.G. Iyer, P.R.G. Varma and V.N. Sankaranarayanan, 1992. Bacterial contamination of mussels at Mahe estuary, Malabar Coast. *Fish. Technol.*, 29: 57-61.

Goyal, S.M., C.P. Gerba and J.L. Melnick, 1979. Human enteroviruses in oysters and their overlying waters. *Appl. Environ. Microbiol.*, 37(3): 572-581.

Grabow, W.O.K., J.C. De Villiers and C.I. Schildhauer, 1992. Comparison of selected methods for the enumeration of faecal coliforms and *Escherichia coli* in shellfish. *Appl. Environ. Microbiol.,* 58(9): 3203-3204.

Grant, J., 1996. The relationship of bioenergetics and the environment to the field growth of cultured bivalves. *J. Exp. Mar. Biol. Ecol.*, 200: 239-256.

Grant, J. and B. Thorpe, 1991. Effects of suspended sediment on growth, respiration and excretion of the soft-shell clam (*Mya arenaria*). *Can. J. Fish. Aquat. Sci.,* 48: 1285-1292.

Grant, J., M. Dowd, K. Thompson, C. Emerson and A. Hatcher, 1993. Perspectives on field studies and related biological models of bivalve growth and carrying capacity. In: Dame, R. (Ed.) Bivalve Filter Feeders and Marine Ecosystem Processes. Vol. G33, NATO ASI Series. Springer-Verlag, Berlin: 371-420.

Griffiths, R.J., 1980. Filtration, respiration and assimilation in the black mussel *Choromytilus meridionalis. Mar. Ecol. Prog. Ser.*, 3: 63-70.

Gruffydd, L.L.D., R. Huxley and D.J. Crisp, 1984. The reduction in growth of *Mytilus edulis* in fluctuating salinity regimes measured using laser diffraction patterns and the exaggeration of this effect by using tap water as the diluting medium. *J. Mar. Biol. Ass. U.K.*, 64: 401-409.

Grundy, M.M., N.A. Ratcliffe and M.N. Moore, 1996. Immune inhibition in marine mussels by polycyclic aromatic hydrocarbons. *Marine Environ. Res.,* 42(1-4): 187-190.

Gundersen, J.S., W.D. Gardner, M.J. Richardson and I.D. Walsh, 1998. Effects of monsoons on the seasonal and spatial distributions of POC and chlorophyll in the Arabian Sea. *Deep-Sea Res. Part 2, Top. Stud. Oceanogr.*, 45(10-11): 2103-2132.

Gupta, T.R.C. and B.K. Bhattacharya, 2003. Effect of sewage on the ecology of Gurpur estuary, Dakshina Kannada, Karnataka. In: Aravindkumar (Ed.) Aquatic Ecosystem. ACH Publishing Corporation, New Delhi, 809-826.

Gupta, T.R.C., S. Nayar, N.V. Yateeshchandra and C. Lingadhal, 1998a. Carbon fixation by phytoplankton in the near-shore waters off Mangalore. *J. Mar. Biol. Ass. India,* 40(1&2): 1-5.

Gupta, T.R.C., R.J. Katti, V. Hariharan, M.S. Satish, G. Gowda and H.P.C. Shetty, 1998b. Ecological monitoring in the Arabian Sea off Mangalore-before and after a decade

receiving effluent. In: M. Mohan Joseph (Ed.). The First Indian Fisheries Forum Proceddings, Asian Fisheries Society, Indian Branch, Mangalore, 295-302.

Harding, J.M., C. Couturier, G.J. Parsons and N.W. Ross, 2004. Evaluation of the neutral red assay as a stress response indicator in cultivated mussels (*Mytilus* spp.) in relation to post-harvest processing activities and storage conditions. *Aquaculture*, 231(1-4): 315-326.

Hawkins, A.J.S. and B.L. Bayne, 1991. Nutrition of marine mussels: factors influencing the relative utilizations of protein and energy. *Aquaculture* 94: 177-196.

Hawkins, A.J.S. and B.L. Bayne, 1992. Physiological processes and the regulation of production. In: Gosling E. (Ed.) The mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Elsevier Science, Amsterdam, 171-222.

Hawkins, C.M., T.W. Rowell and P. Woo, 1987. The importance of cleansing in the calculation of condition index in the soft-shell clam, *Maya arenaria* (L.). *J. Shellfish Res.*, 6: 29-36.

Hawkins, A.J.S., B.L. Bayne, S. Bougrier, M. Héral, J.I.P. Iglesias, E. Navarro and R.F.M. Smith, 1998. Some general relationships in comparing the feeding physiology of suspension-feeding bivalve molluscs. *J. Exp. Mar. Biol.*, 219: 87-103.

Hawkins, A.J.S., M.R. James, R.W. Hickman, S. Hatton and M. Weatherhead, 1999. Modelling of suspension feeding and growth of the green-lipped mussel *Perna canaliculus* exposed to natural and experimental variation of seston availability in the Marlborough Sounds, New Zealand. *Mar. Ecol. Prog. Ser.*, 191: 217-232.

Hernroth, B.E., A.C.C. Hansson, A.S.R. Holm, R.Girones and A.K. Allard, 2002. Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, *Mytilus edulis*: the First Scandinavian Report. *Appl. Environ. Microbiol.*, 68(9): 4523-4533.

Hickman, R.W. and J. Illingworth, 1980. Condition cycle of the green-lipped mussel *Perna canaliculus* in New Zealand. *Mar. Biol.*, 60: 27-38.

Hickman, R.W., R.P. Waite, J. Illingworth, J.L.M.Young and G. Payne, 1991. The relationship between farmed mussels, *Perna canaliculus* and available food in Pelorus-Kenepuru Sound, New Zealand. *Aquaculture*, 99: 49-68.

Hilbish, T.J. and K.M. Zimmerman, 1988. Genetic and nutritional control of the gametogenic cycle in *Mytilus edulis*. *Mar. Biol.*, 98: 223-228.

His, E., R. Beiras and M.N.L. Seaman, 2000. The assessment of marine pollution-bioassays with bivalve embryos and larvae. In: Southward, A.J., Tyler, P.A., Young, C.M. (Eds.) Advances in *Mar. Biol.*, vol. 37, Academic Press, San Diego, 1-178.

Hoff, J.C. and R.C. Becker, 1968. The accumulation and elimination of crude and clarified polio virus suspensions by shellfish. *Amer. J. Epidemiol.*, 90: 53-61.

Hofmann, E.E., J.M. Klinck, E.N. Powell, S. Boyles and M. Ellis, 1994. Modeling oyster populations II. Adult size and reproductive effort. *J. Shellfish. Res.*, 13: 165-182.

Hogg, I.D. and R.H. Norris, 1991. Effects of runoff from land clearing and urban development on the distribution and abundance of macroinvertebrates in pool areas of a river. *Aust. J. Mar. Freshw. Res.*, 42: 507-518.

Hood, M.A. and G.E. Ness, 1982. Survival of *Vibrio cholerae* and *Escherichia coli* in estuarine waters and sediments. *Appl. Environ. Microbiol.*, 43(3): 578-584.

Hood, M.A., G.E. Ness and N.J. Blake, 1983. Relationship among faecal coliforms, *Escherichia coli* and *Salmonella* spp. in shellfish. *Appl. Environ. Microbiol.*, 45(1): 122-126.

Howell, J.M., M.S. Coyne and P. Cornelius, 1995. Faecal bacteria in agricultural waters of the Bluegrass region of Kentucky. *J. Environ. Qual.*, 24: 411-419.

Hung, T.C., P.J. Meng, B.C. Han, A. Chuang and C.C. Huang, 2001. Trace metals in different species of mollusca, water and sediments from Taiwan coastal area. *Chemosphere*, 44(4): 833-841.

Iglesias, J.I.P., A.P. Camacho, E. Navarro, U. Labarta, R. Beiras, A.J.S. Hawkins and J. Widdows, 1996. Microgeographic variability in feeding, absorption and condition of mussels (*Mytilus galloprovincialis* Lmk.): A transplant experiment. *J. Shellfish Res.*, 15: 673-680.

Incze, L.S., R.A., Lutz and L. Watling, 1980. Relationships between effects of environmental temperature and seston on growth and mortality on *Mytilus edulis* in a temperate Northern Estuary. *Mar. Biol.*, 57: 147-156.

Inglis, G.J., B.J. Hayden and A.H. Ross, 2000. An overview of factors affecting the carrying capacity of coastal embayments for mussel culture. NIWA Client Report: CHC00/69, 30 pp.

Isono, R.S., J. Kita and C. Kishida, 1998. Upper temperature effect on rates of growth and oxygen consumption of the Japanese littleneck clam *Ruditapes philippinaru. Nippon Suisan Gakkaishi,* 64(3): 373-376.

Jackson, K.L. and D.M. Ogburn, 1999. Review of depuration and its role in shellfish quality assurance. Final Report to Fisheries Research and Development Corporation. Project No. 96/355.

James, P.S.B.R., 1992. Impact of fishing along the west coast of India during southwest monsoon period on the finfish and shellfish resources and the associated management considerations. *Bull. Cent. Mar. Fish. Res. Inst.*, 45: 251-259.

Jaramillo, R. and J. Navarro, 1995. Reproductive cycle of the Chilean ribbed mussel *Aulacomya ater* (Molina, 1782). *J. Shellfish Res.*, 14(1): 165-171.

Joseph, T., K.K. Balachandran, M. Nair and V.N. Sankaranarayanan, 1998. Hydrochemical studies along the coastal waters off Mangalore. *Ecol. Env. Cons.*, 4(1-2): 9-12.

Julshamn, K., 1981. Studies on major and minor elements in molluscs in Western Norway. VII. The contents of 12 elements, including copper, zinc, cadmium and lead in common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) relative to the distance from the industrial sites in Sørfjorden, inner Hardangerfjorden. *Fiskeridirektoratets Skrifter, Serie Ernoering.*, 1:267-287.

Kadhim, M.A., 1990. Methodologies for monitoring the genetic effects of mutagens and carcinogens accumulated in the body tissues of marine mussels. *Rev. Aquat. Sci.*, 2(1): 83-107.

Kan-atireklap, S., S. Tanabe, J. Sanguansin, M.S. Tabucanon and M. Hungspreugs, 1997. Contamination by butyltin compounds and organochlorine residues in green mussel (*Perna viridis*, L.) from Thailand coastal waters. *Environ. Pollut.*, 97(1-2): 79-89.

Kannan, K., S. Tanabe and R. Tatsukawa, 1995. Geographical distribution and accumulation features of organochlorine residues in fish in tropical Asia and Oceania. *Environ. Sci. Technol.*, 9: 2673-2683.

Kaplunovsky, A.S., 2005. Factor analysis in environmental studies. *HAIT J. of Sci. Eng.*, B, 2(1-2): 54-94.

Karunasagar, I., H.S.V. Gowda, M. Subburaj, M.N. Venugopal and I. Karunasagar, 1984. Outbreak of paralytic shellfish poisoning in Mangalore west coast of India. *Curr. Sci.*, 53(5): 247-249.

Karunasagar, I., S. Mathew and I. Karunasagar, 1987. *Vibrio vulnificus* in fish and clams from Mangalore waters. *Indian J. Mar. Sci.*, 16: 136-137.

Karunasagar, I., P.M. Suseela, G.R. Malathi and I. Karunasagar, 1990. Incidence of human pathogenic vibrios in seafoods harvested along the coast of Karnataka (India). *FAO Fish Rep.*, 401 (Suppl.), 53-56.

Karunasagar, I., G. Sugumar, I. Karunasagar and A. Reilly, 1995. Rapid detection of *Vibrio cholerae* contamination of seafood by polymerase chain reaction. *Mol. Mar. Biol. Biotechnol.*, 4: 365-368.

Karunasagar, I., G. Sugumar, I. Karunasagar and A. Reilly, 1996. Rapid polymerase chain reaction method for detection of Kanagawa positive *Vibrio parahaemolyticus* in seafoods. *Int. J. Food Microbiol.*, 31: 317-323.

Karunasagar, I., B. Joseph, K.K. Philipose and I. Karunasagar, 1998. Another outbreak of PSP in India. *Harmful Algae News*, 17: 1.

Kautsky, N., 1982. Quantitative studies on the gonad cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. *Mar. Biol.*, 68: 143-160.

Kelsey, H., D.E. Porter, G. Scott, M. Neet and D. White, 2004. Using geographic information systems and regression analysis to evaluate relationships between land use and faecal coliform bacterial pollution. *J. Exp. Mar. Biol. Ecol.*, 298(2): 197-209.

Kenyon, J.E., D.R. Piexoto, B. Austin and D.C. Gillies, 1984. Seasonal variation in numbers of *Vibrio cholerae* (non-01) isolated from California coastal waters. *Appl. Environ. Microbiol.*, 47(6): 1243-1245.

Kobayashi, M., E.E. Hofmann, E.N. Powell, J.M. Klinck and K. Kusaka, 1997. A population dynamics model for the Japanese oyster, *Crassostrea gigas*. *Aquaculture*, 149: 285-321.

Kripa, V. and K.K. Appukuttan, 2003. Marine Bivalves. In: M.M. Joseph and Jayaprakash, A.A. (Eds.) *Status of Exploited Marine Fishery Resources* of India. Central Marine Fisheries Research Institute, Kochi, India: 211-220.

Krishnakumar, P.K. and V.K. Pillai, 1990. Mercury near a caustic soda plant at Karwar, India. *Mar. Pollut. Bull.*, 21: 304-307.

Krishnakumar, P.K. and G.S. Bhat, 2007. Seasonal and interannual variations of oceanographic conditions off Mangalore coast (Karnataka, India) in the Malabar upwelling system during 1995-2004 and their influences on pelagic fishery. *Fish. Oceanogr., (in press)*.

Krishnakumar, P.K., V.K. Pillai and K.K. Valsala, 1990a. Bioaccumulation of trace metals by marine flora and fauna near a caustic soda plant (Karwar, India). *Indian J. Fish.*, 37: 129-137.

Krishnakumar, P.K., P.K. Asokan and V.K. Pillai, 1990b. Physiological and cellular responses to copper and mercury in the green mussel *Perna viridis* (Linnaeus). *Aquat. Toxicol.*, 18(3): 163-173.

Krishnakumar, P.K., E. Casillas and U.Varanasi, 1994. Effects of environmental contaminants on the health of *Mytilus edulis* from Puget sound, Washington, USA. I. Cytochemical measures of lysosomal responses in the digestive cells using automatic image analysis. *Mar. Ecol. Prog. Ser.*, 106: 249-261.

Krishnakumar, P.K., G.S. Bhat and M. Rajagopala, 2004a. Monitoring the concentrations of cadmium, lead and zinc from the coastal waters of India using voltametric technique. International Workshop on Marine Pollution and Ecotoxicology, NIO, Goa, India and IWA, UK, extended abstracts, 25-26 February, 2004, 148-150.

Krishnakumar, P.K., G. Sasikumar, G.S. Bhat and P.K. Asokan, 2004b. Biomarkers of environmental contaminants in field population of green mussel (*Perna viridis*) from Karnataka-Kerala coast. International workshop on marine pollution and Ecotoxicology. NIO, Goa, India and IWA, UK, extended abstracts, 25-26 February, 2004, 198-199.

Krishnakumar, P.K., G.S. Bhat, N.G. Vaidya and V.K. Pillai, 1998. Heavy metal distribution in the biotic and abiotic matrices along Karnataka coast, west coast of India. *Indian J. Mar. Sci.*, 27(1): 201-205.

Kureishy, T.W., S. Sanzgiry and A. Baraganga, 1981. Some heavy metals in fishes from the Andaman Sea. *Indian J. Mar. Sci.*, 19: 303-307.

Kureishy, T.W., S. Sanzgiry, M.D. George and A. Baraganga, 1983. Mercury, cadmium and lead in different tissues of fishes and zooplankton from the Andaman Sea. *Indian J. Mar. Sci.*, 12: 60-63.

Kuriakose, P.S., 1980. Mussels (Mytilidae: Genus *Perna*) of the Indian coast. In: *Coastal Aquaculture: Mussel Farming, Progress and Prospects. Cent. Mar. Fish. Res. Inst. Bull.*, 29: 1-5.

Labelle, R.L., C.P. Gerba, S.M. Goyal, J.L. Melnick, I. Cech and G.F. Bogdan, 1980. Relationships between environmental factors, bacterial indicators and the occurrence of enteric viruses in estuarine sediments. *Appl. Environ. Microbiol.*, 39(3): 588-596.

Lai, K.P., W. Li, Y. X.u, M.H. Wong and C.K.C. Wong, 2004. Dioxinlike components in human breast milk collected from Hong Kong and Guangzhou. *Environ. Res.*, 96: 88-94.

Lakshmanan, P.T. and P.N.K. Nambisan, 1983. Seasonal variations in trace metal content in bivalve mollusks, *Villorita cyprinoides* var. *cochinensis* (Hanley), *Meretrix casta* (Chemnitz) and *Perna viridis* (Linnaeus). *Indian J. Mar. Sci.*, 12: 100-103.

Lakshmipathi, M.T., 2001. Spatial and temporal distribution of benthos in relation to sediment characteristics in the inshore water of Arabian Sea off Mangalore. Ph.D Thesis Univ. Agril. Sci., Bangalore, 187 pp.

Lam, P.K.S. and R.S.S. Wu, 2003. Use of biomarkers in environmental monitoring. Scientific and Technical Advisory Panel (STAP) Workshop on *The use of bioindicators, biomarkers and analytical methods for the analysis of POPs in developing countries*, 10-12, December 2003, 79 pp.

Langdon, J.L. and R.I.E. Newell, 1990. Utilization of detritus and bacteria as food sources by two bivalve suspension-feeders, the oyster *Crassostrea virginica* and the mussel *Geukensia demissa. Mar. Ecol. Prog. Ser.*, 58: 299-310.

Lares, M.L., L.E. Rivero and M.A.H. Diaz, 2005. Cd concentration in the tissue vs. the nacreous layer of *Mytilus californianus. Mar. Pollut. Bull.*, 50: 1373-1381.

Latasa, M. and R.R. Bidigare, 1998. A comparison of phytoplankton populations of the Arabian Sea during the spring inter-monsoon and southwest monsoon of 1995 as described by HPLC-analyzed pigments. *Deep-Sea Res. Part 2, Top. Stud. Oceanogr.*, 45(10-11): 2133-2170.

Lawrence, D.R. and G.I. Scott, 1982. The determination and use of condition index of oysters. *Estuaries*, 5: 23-27.

Leavitt, D.F., B.A. Lancaster, A.S. Lancaster and J.M. Capuzzo, 1990. Changes in the biochemical composition of a subtropical bivalve, *Arca zebra*, in response to contaminant gradients in Bermuda. *J. Exp. Mar. Biol. Ecol.*, 138: 85-98.

Lee, S.Y., 1985. Population dynamics of the green mussel *Perna viridis* (L.) (Bivalvia: Mytilacea) in Victoria Harbour, Hong Kong: dominance in a polluted environment. *Asian Mar. Biol.*, 2: 107-118.

Lee, S.Y., 1986. Growth and reproduction of the green mussel *Perna viridis* (L.) (Bivalvia: Mytilacea) in contrasting environments in Hong Kong. *Asian Mar. Biol.*, 3: 111-127.

Lees, D., 2000. Viruses and bivalve shellfish. *Int. J. Food Microbiol.*, 59: 81-116.

Legnani, P., E. Leoni, D. Lev, R. Rossi, G.C. Villa and P. Bisbini, 1998. Distribution of indicator bacteria and bacteriophages in shellfish and shellfish-growing waters. *J. Appl. Microbiol.*, 85: 790-798.

Lesser, M. P., J.D. Witman and K.P. Sebens, 1994. Effects of flow and seston availability on scope for growth of benthic suspension-feeding invertebrates from the Gulf of Maine. *Biol. Bull.*, 187: 319-335.

Leverone, J.R., 1995. Growth and survival of caged adult bay scallops (*Argopecten irradians concentricus*) in Tampa Bay with respect to levels of turbidity, suspended solids and chlorophyll a. *Florida Sci.*, 58: 216-227.

Li, Y.F., 1999. Global technical hexachlorocyclohexane usage and its contamination consequences in the environment: from 1948 to 1947. *Sci. Total Environ.*, 232: 121-158.

Lingadhal, C., 1991. Ecological monitoring of the inshore waters of Mangalore. M.F.Sc. Thesis. Univ. Agrl. Sci. Bangalore, 126 pp.

Lingadhal, C., 1995. Primary production in the Arabian Sea off Mangalore. Ph.D. Thesis, Univ. Agrl. Sci, Bangalore, 138 pp.

Lingadhal, C., T.R.C. Gupta and A.M. Ramesh, 2003. Distribution of chlorophyll pigments in the Arabian Sea off Mangalore (west coast of India) in relation to nutrients. *J. Mar. Biol. Ass. India,* 45(2): 121-127.

Lipp, E.K., R. Kurz, R. Vincent, C.R. Palacios, S.R. Farrah and J.B. Rose, 2001b. The effects of seasonal variability and weather on faecal pollution and enteric pathogens in a subtropical estuary. *Estuaries*, 24(2): 266-276.

Lipp, E.K., S.A. Farrah and J.B. Rose, 2001a. Assessment and impact of microbial faecal pollution and human enteric pathogens in a coastal community. *Mar. Pollut. Bull.*, 42(4): 286-293.

Lloyd, D.S., 1987. Turbidity as a water quality standard for salmonid habitats in Alaska. *North Am. J. Fish. Manage.*, 7: 34-45.

Lobel, P.B., P. Mogie, D.A. Wright and B.L. Wu, 1982. Metal accumulation in four molluscs. *Mar. Pollut. Bull.*, 13:170-174.

Lowe, D.M. and R.K. Pipe, 1994. Contaminant induced lysosomal membrane damage in marine mussel digestive cells: an *in vitro* study. *Aquat. Toxicol.*, 30(4): 357-365.

Lowe, D.M., M.N. Moore and B.M. Evans, 1992. Contaminant impact on interactions of molecular probes with lysosomes in living hepatocytes from dab *Limanda limanda*. *Mar. Ecol. Prog. Ser.*, 91: 135-140.

Lowe, D.M., V.U. Fossato and M.H. Depledge, 1995a. Contaminant-induced lysosomal membrane damage in blood cells of mussels *Mytilus galloprovincialis* from the Venice Lagoon: an *in vitro* study. *Mar. Ecol. Prog. Ser.*, 129: 189-196.

Lowe, D.M., C. Soverchia and M.N. Moore, 1995b. Lysosomal membrane responses in the blood and digestive cells of mussels exposed to fluoranthene. *Aquat. Toxicol.*, 33: 105-112.

Lubet, P., 1981. Action de la temperature sur le cycle de reproduction des lamellibranches. *Bull. Soc. Zool. France,* 106: 283-292.

Lucas, A. and P.G. Beninger, 1985. The use of physiological condition index in marine bivalve aquaculture. *Aquaculture,* 44: 187-200.

Luis, E.S., A.H. Balagot, A.C. Villaflor, F.C. Sanchez and E.N. Develles, 1989. Pb, Cd and Hg contents of bivalves collected during the different seasons of the year. *Food Chem.*, 32(4): 239-255.

MacDonald, B.A., Bacon, G.S. and Ward, J.E., 1998. Physiological response of infaunal (*Mya arenaria*) and epifaunal (*Placopecten magellanicus*) bivalves to variations in the concentration and quality of suspended particles II. Absorption efficiency and scope of growth. *J. Exp. Mar. Biol. Ecol.,* 219: 127-141.

Macfarlane, S.L., 1996. Shellfish as the Impetus for Embayment Management. *Estuaries,* 19(2A): 311-319. (*http://estuaries.ole miss.edu/cdrom/ESTU 1996_19_2A_311_*, 319.pdf).

Maguire, J.A., P.G. Fleury and G.M. Burnell, 1999. Some methods for quantifying quality in the scallop *Pecten maximus* (L). *J. shellfish Res.*, 18(1): 59-66.

Mallin, M.A., S.H. Ensign, M.R. McIver, G.C. Shank and P.K. Fowler, 2001. Demographic, landscape and meteorological factors controlling the microbial pollution of coastal waters. *Hydrobiologia*, 460: 185-193.

Mamaca, E., R.K. Bechmann, S. Torgrimsen, E. Aas, A. Bjørnstad, T. Baussant and S.L. Floch, 2005. The neutral red lysosomal retention assay and comet assay on haemolymph cells from mussels (*Mytilus edulis*) and fish (*Symphodus melops*) exposed to styrene. *Aquat. Toxicol.,* 75(3): 191-201.

Manjappa, K., 1987. Distribution of chlorophyll and phaeopigments in relation to hydrographical parameters in the Arabian Sea off Mangalore. M.F.Sc. Thesis. Univ. Agrl. Sci. Bangalore, 133 pp.

Marigómez, I. and L.B. Villacorta, 2003. Pollutant-specific and general lysosomal responses in digestive cells of mussels exposed to model organic chemicals. *Aquat. Toxicol.*, 64(3): 235-257.

Marino, A., L. Lombardo, C. Fiorentino, B. Orlandella, L. Monticelli, A. Nostro and V. Alonzo, 2005. Uptake of *Escherichia coli*, *Vibrio cholerae* non-01 and *Enterococcus durans* by and depuration of mussels (*Mytilus galloprovincialis*) *Int. J. Food Microbiol.,* 99: 281-286.

Martin, M., 1985. State Mussel Watch: Toxics Surveillance in California. *Mar. Pollut. Bull.,* 16(4): 140-146.

Martin, N., G. Ichikawa, J. Goetyl, M. Reyes and M.D. Stevenson, 1984. Relationships between physiological stress and trace toxic substances in the Bay mussel, *Mytilus edulis* from San Francisco Bay, California. *Mar. Environ. Res.,* 11: 91-110.

Masilamoni, J.G., K.S. Jesudoss, K. Nandakumar, K.K. Satpathy, J. Azariah and K.V.K. Nair, 1997. Physiological responses of the green mussel *Perna viridis* in relation to size and salinity. *Proc. Indian Natu. Sci. Acad.,* B 63: 305-314.

Mendon, M.R., R.J. Katti, K.M. Rajesh and T.R.C Gupta, 2002. Distribution and abundance of copepods and copepodites in the Arabian Sea off Mangalore receiving treated industrial effluents *Poll. Res.*, 21(1): 45-49.

Menon, N.R., T.R.C. Gupta, V. Hariharan, R.J. Katti and H.P.C. Shetty, 1977. Marine plankton of Mangalore waters-A pre-pollution assessment. *Proc. Symp. Warm Water Zoopl. Spl.,* Publ. UNESCO/ NIO, 274-283.

Meyer, A. and R. Neeb, 1983. Determination of Co and Ni by adsorption voltammetry in supporting electrolytes containing triethanolamine and dimethylglyoxime. *Anal. Chem.*, 315: 118-120.

Mohamed, K.S., C. Muthiah, D. Nagaraja and G. Sampathkumar, 1998. Initiation of marine mussel culture activities in Dakshina Kannada district, Karnataka. *Mar. Fish. Infor. Serv., T& E. Ser.*, 155: 10-15.

Mohankumar, M.B., R.J. Katti, K.S.V. Moorthy and R.K. D'Souza, 2003. Selected heavy metals in the sediment and macrobenthos of the coastal waters off Mangalore. *Indian J. Fish.*, 50(2): 263-268.

Monirith, I., D. Ueno, S. Takahashi, H. Nakata, A. Sudaryanto, A. Subramanian, S. Kauppiah, A. Ishmail, M. Muchtar, J. Zheng, B.J. Richardson, M. Prudente, N.D. Hue, T.S. Tana, A.V. Tkalin and S. Tanabe, 2003. Asia-Pacific mussel watch: monitoring contamination of persistant organochlorine compounds in coastal waters of Asian countries. *Mar. Pollut. Bull.*, 46: 281-300.

Montaudouin, X., 1996. Factors involved in growth of plasticity of cockles *Cerastoderma edule* (L.), identified by field survey and transplant experiments. *J. Sea Res.*, 36 (3-4): 251-265.

Moore, M.N., 1976. Cytochemical demonstration of latency of lysosomal hydrolases in digestive cells of the common mussel, *Mytilus edulis* and changes induced by thermal stress. *Cell Tissue Res.*, 175: 279-287.

Moore, M.N., 1980. Cytochemical determination of cellular responses to environmental stressors in marine organisms. In: A.D. McIntyre and J.B. Pearce (Eds.) Biological effects of marine pollution and the problems of monitoring organisms, *Rapp. P.V. Reun. Cons. Int. Exp. Mer.*, 179: 7-15.

Moore, M.N. and K.R. Clarke, 1982. Use of microstereology and quantitative cytochemistry to determine the effects of crude oil-derived aromatic hydrocarbons on lysosomal structure and function in a marine bivalve mollusc, *Mytilus edulis*, *Histochem. J.*, 14: 713.

Moore, M.N. and D.M. Lowe, 1985. Cytological and cytochemical measurements. In: B.I. Bayne (Ed.) The effects of Stress and Pollution on Marine Animals, Praeger Scientific, New York, 46-74.

Moore, M.N., J. Widdows, J.J. Cleary, R.K. Pipe, P.N. Salkeld, P. Donkin, S.V. Farrar, S.V. Evans and P.E. Thomson, 1984. Responses of the mussel *Mytilus edulis* to copper and phenanthrene: interactive effects. *Mar. Environ. Res.*, 14: 167-183.

Moore, N.M., C. Soverchia and M. Thomas, 1996a. Enhanced lysosomal autophagy of intracellular proteins by xenobiotics in living molluscan blood cell. *Acta Histochemical et Cytochemica*, 29: 947-948.

Moore, M.N., R.J. Wedderburn, D.M. Lowe and M.H. Depledge, 1996b. Lysosomal reaction to xenobiotics in mussel haemocytes using BODIPY-FL-verapamil. *Marine Environ. Res.*, 42(1-4): 99-105.

Moore, M.N., D.M. Lowe, C. Soverchia, S.D. Haigh and S.G. Hales, 1997. Uptake of a non-calorific, edible sucrose polyester oil and olive oil by marine mussels and their influence on uptake and effects of anthracene. *Aquat. Toxicol.*, 39(3-4): 307-320.

Moore, M.N., T. Wade, R.J. Wedderburn, D.M. Lowe, G. Balashov, H. Buyukgun-gor, Y. Daurova, Y. Denga, E. Kostylev, P.E. Mihnea, S. Moncheva, S. Tabagari, C. Ciocan, H. Ozkoc and M.H. Depledge, 1999. The UNESCO/IOC "Black Sea Mussel Watch Pilot Study": Biological effects and contaminant residues. In: Black Sea Pollution

Assessment, Black Sea Environmental Series, United Nations Publications, New York 10: 279-292.

Morse, D.L., J.J. Guzewich, J.P. Hanrahan, R. Stricof, M. Shayegani, R. Deibel, J.C. Grabau, N.A. Nowak, J.E. Herrmann, G. Cukor and N.R. Blacklow, 1986. Widespread outbreaks of clam and oyster-associated gastroenteritis. Role of Norwalk virus. *New England J. Medicine*, 314(11): 678-681.

Morton, B. 1987. The functional morphology of the organs of the mantle cavity of *Perna viridis* (Linnaeus, 1758) (Bivalvia: Mytilacea). *Amer. Malacol. Bull.*, 5: 159-164.

Mujika, M.I., R. Girones, G.T. Quesada, M. Calvo and F. Lucena, 2002. Depuration dynamics of viruses in shellfish. *Int. J. Food Microbiol.*, 77: 125-133.

Muraleedharan, P.M. and S.P. Kumar, 1996. Arabian Sea upwelling - A comparison between coastal and open ocean regions. *Curr. Sci.*, 71: 842-846.

Muralidharan, G. and P.V. Raja, 1997. Trace element concentration in the meat of the edible clam *Marcia recens* Chemnitz (Pelecypoda: Veneridae). *Indian J. Mar. Sci.*, 26(4): 383-385.

Myint, U.M. and P.A. Tyler, 1982. Effects of temperature, nutritive and metal stressors on the reproductive biology of *Mytilus edulis*. *Mar. Biol.*, 67: 209-223.

Nagabhushanam, R. and V.S. Mane, 1975. Reproduction in the mussel, *Mytilus viridis* at Ratnagiri. *Bull. Dept. Mar. Sci.*, Univ. Cochin 2: 377-387.

Nair, K.V.K., 1984. Metals are marine pollutants. Transactions of the Indian Institute of Metals, 37(6): 657-663.

Nair, M.N. and K.K. Appukuttan, 2003. Effect of temperature on the development, growth, survival and settlement of green mussel, *Perna viridis* (Linnaeus, 1758). *Aquac. Res.*, 34: 1037-1045.

Nair, P.V. 1974. Studies of primary production in the Indian Seas. Ph.D. Thesis, Cochin University, Kerala.

Narasimham, K.A., 1980. Fishery and biology of the green mussel, *Perna viridis* (Linnaeus). In: Nayar K.N., Mahadevan S., Alagarswami K. and Sundaram P.T.M. (Eds) *Coastal Aquaculture: Mussel Farming, Progress and Prospects,* Central Marine Fisheries Research Institute, Cochin, India, 10-17.

Nasci, C., L. Da Ros, G. Campesan and V.U. Fossato, 1998. Assessment of the impact of chemical pollutants on mussel, *Mytilus galloprovincialis*, from the Venice Lagoon, Italy. *Mar. Environ. Res.*, 46(1-5): 279-282.

Navarro, E., J.I.P. Iglesias, A.P. Camacho, U. Labarta and R. Beiras, 1991. The physiological energetics of mussels (*Mytilus galloprovincialis* Lmk) from different cultivation rafts in the Ria de Arosa (Galicia, N.W. Spain). *Aquaculture*, 94: 197-212.

Nayar, K.N. and K.S. Rao, 1985. Molluscan fisheries of India. *Mar. Fish. Infor. Serv. T&E Ser.*, 61: 1-7.

NCDEHNR, 1994. Water quality progress in North Carolina 1992-1993, 305(b) North Carolina Department of Environment, Health and Natural Resources, Division of Environmental Management, Water Quality Section, Raleigh, North Carolina. Report, No. 94-07.

NCDEM, 1990. North Carolina Coastal Marinas: Water Quality Assessment. North Carolina Division of Environmental Management, Raleigh, NC. Report No. 90-01.

NCDEM, 1991. Coastal Marinas: Field Survey of Contaminants and Literature Review. North Carolina Division of Environmental Management, Raleigh, NC. Report No. 91-03.

Neill, M., 2004. Microbiological indices for total coliform and *E. coli* bacteria in estuarine waters. *Mar. Pollut. Bull.*, 49: 752-760.

Nesto, N., M. Bertoldo, C. Nasci and L. Da Ros, 2004. Spatial and temporal variation of biomarkers in mussels (*Mytilus galloprovincialis*) from the Lagoon of Venice, Italy. *Mar. Environ. Res.*, 58(2-5): 287-291.

Newcombe, C.P. and D.D. MacDonald, 1991. Effects of suspended sediment on aquatic ecosystems. *North Am. J. Fish. Manage.*,11: 72-82.

Newell, R.C., 1979. The biology of intertidal animals. Marine Ecological Surveys, Faversham, UK, 781 pp.

Newell, R.I.E., 1985. Physiological effects of the MSX parasite *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) on the American oyster *Crassostrea virginica* (Gmelin). *J. Shellfish Res.*, 5: 91-95.

Newell, R.I.E., T.J. Hilbish, R.K. Koehn and C.J. Newell, 1982. Temporal variation in the reproductive cycle of *Mytilus edulis* L. (Bivalvia, Mytilidae) from localities on the east coast of the U.S.A. *Biol. Bull.*, 162: 299-310.

Nicholson, S. and P. Szefer, 2003. Accumulation of metals in the soft tissues, byssus and shell of the mytilid mussel *Perna viridis* (Bivalvia: Mytilidae) from polluted and uncontaminated locations in Hong Kong coastal waters. *Mar. Pollut. Bull.*, 46(8): 1040-1043.

Nicholson, S., 1999. Cytological and physiological biomarker responses from green mussels, *Perna viridis* (L.) transplanted to contaminated sites in Hong Kong coastal waters. *Mar. Pollut. Bull.*, 39: 261-268.

Nigro, M., A. Falleni, I.D. Barga, V. Scarcelli, P. Lucchesi, F. Regoli and G. Frenzilli, 2006. Cellular biomarkers for monitoring estuarine environments: Transplanted versus native mussels. *Aquat. Toxicol.*, 77(4): 339-347.

NSSP (National Shellfish Sanitation Program), 1999. *Guide for the Control of Molluscan Shellfish, Model Ordinance*. Chapter IV. U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Centre for Food Safety and Applied Nutrition, Washington, D.C, USA (*available at http://vm.cfsan.fda.gov/~ear/nsspotoc.html*).

O'Connor, T., 1992. Recent trends in coastal environmental quality: Results from the first five years of the NOAA Mussel Watch Project. NOAA, Department of Commerce, 1305 East-West Highway, Silver Spring, MD 20910, 46 pp.

O'Connor, T.P., 1996. Trends in chemical concentrations in mussels and oysters collected along the US coast from 1986-1993. *Mar. Environ. Res.*, 41: 183-200.

Ojea, J., A.J. Pazos, D. Martinez, S. Novoa, J.L. Sanchez and M. Abad, 2004. Seasonal variation in weight and biochemical composition of the tissues of *Ruditapes decussates* in relation to the gametogenic cycle. *Aquaculture*, 238: 451-468.

Okay, O.S., L.Tolun, V.Tüfekçi, F. Telli-Karakoç and P. Donkin, 2006. Effects of pyrene on mussels in different experimental conditions. *Environ. Int.*, 32(4): 538-544.

Padiyar, A. (1999). Studies on the suspended particulate matter in the Mulki estuary, Dakshina Kannada district. M.F.Sc., Thesis Univ. Agril. Sci., Bangalore, 74pp.

Page, H.M. and D.M. Hubbard, 1987. Temporal and spatial patterns of growth in mussels *Mytilus edulis* on an offshore platform: relationships to water temperature and food availability. *J. Exp.Mar. Biol. Ecol.*, 111: 159-179.

Pan, J. F. and W.X. Wang, 2004. Influences of dissolved and colloidal organic carbon on the uptake of Ag, Cd and Cr by the marine mussel *Perna viridis*. *Environ. Pollut.*, 129(3): 467-477.

Pandit, G.G., S.K. Sahu, S. Sharma and V.D. Puranik, 2006. Distribution and fate of persistent organochlorine pesticides in coastal marine environment of Mumbai. *Environ. Int.*, 32(2): 240-243.

Pant, A., 1992. Primary productivity in coastal and off-shore waters of India during two southwest monsoons, 1987 and 1989. Oceanography of the Indian Ocean. Desai, B.N. (Ed.) New Delhi, India. Oxford and IBH: 81-90.

Parulekar, A.H., S.G. Dalal, Z.A. Ansari and S.N. Harkantra, 1982. Environmental physiology of raft-grown mussels in Goa, India. *Aquaculture*, 29: 83-93.

Parvathi, A., H.S. Kumar, I. Karunasagar and I. Karunasagar, 2005. Study of the occurrence of *Vibrio vulnificus* in oysters in India by polymerase chain reaction (PCR) and heterogeneity among *V. vulnificus* by randomly amplified polymorphic DNA PCR and *gyrB* sequence analysis. *Environ. Microbiol.*, 7: 995-1002.

Patel, B. and J.P. Chandy, 1988. Mercury in the biotic and abiotic matrices along Bombay Coast. *Indian J. Mar. Sci.*, 17: 55-58.

Payment, P., A. Berte, M. Prévost, B. Ménard and B. Barbeau, 2000. Occurrence of pathogenic microorganisms in the Saint Laurence River (Canada) and comparison of health risks for populations using it as their source of drinking water. *Can. J. Microbiol.*, 46: 565–576.

Paynter, K.T. and E.M. Buresson, 1991. Effects of *Perkinsus marinus* infection in the eastern oyster, *Crassostrea virginica*: II Disease development and impact on the growth rate at different salinities. *J. Shellfish Res.*, 10: 424-431.

Pazos, A.J., G. Roman, C.P. Acosta, M. Abad and J. L. Sanchez, 1997. Seasonal changes in condition and biochemical composition of the scallop Pecten maximus L. from suspended culture in the Ria de Arousa (Galicia, N.W. Spain) in relation to environmental conditions. *J. Exp. Mar. Biol. Ecol.*, 211: 169-193.

Peerzada, N., L. Mcmorrow, S. Skiliros, M. Guinea and P. Ryan, 1990. Distribution of heavy metals in gove harbours. *Sci. of Total Environ.*, 92: 1-12.

Pettibone, G.W., A.S. Sullivan and M.P. Shiaris, 1987. Comparative survival of antibiotic-resistant and sensitive faecal indicator bacteria in estuarine water. *Appl. Environ. Microbiol.*, 53(6): 1241-1245.

Phillips, D.J.H., 1977. The use of biological indicator organisms to monitor metal pollution in marine and estuarine environments-A review. *Environ. Pollut.*, 13: 281-317.

Phillips, D.J.H., 1985. Organochlorines and trace metals in green-lipped mussels *Perna virdis* from Hong Kong waters: a test of indicator ability. *Mar. Ecol. Prog. Ser.*, 21(3): 251-258.

Phillips, D.J.H. and K. Muttarasin, 1985. Trace metals in bivalve molluscs from Thailand. *Marine Environ. Res.*, 15(3): 215-234.

Pickwell, G.V. and S.A. Steinert, 1984. Serum biochemical and cellular responses to experimental cupric ion challenge in mussels. *Mar. Pollut. Res.*, 14: 245-265.

Pillai, C.T. 1980. Microbial flora of mussels in the natural beds and farms. In Mussel Farming-Progress and Prospects. *Bull. Cent. Mar. Fish. Res. Inst.*, 29: 41-43.

Pillai, V.K., A.G. Ponniah, K.K. Valsala and P.V.R. Nair, 1986. Heavy metal load in a few bivalves from coastal waters of south India. In: *Proc. Natl. Sem. on Mussel Watch.* Cochin Univ. of Sci. and Tech., Cochin, 93-104.

Pillai, V.N., M. Devaraj and E. Vivekanandan, 1997. Fisheries environment in the APFIC region with particular emphasis on the northern Indian Ocean. In. Devaraj M. and P. Martosubroto (Eds.) Small pelagic resources and their fisheries in the Asia-Pacific region. Proc. of the first session of the APFIC Working Party on Marine Fisheries Bangkok, Thailand, 13-16 May 1997, 381-424.

Pillai, V.N., V.K. Pillai, C.P. Gopinathan and A. Nandakumar, 2000. Seasonal variations in the physico-chemical and biological characteristics of the eastern Arabian Sea. *J. Mar. Biol. Ass. India,* 42(1&2): 1-20.

Pitt, R., 2000. Source Characterization In: *Innovative Urban Wet-Weather Flow Management Systems*. J.P. Heaney, R. Pitt and R. Field. Publication No. EPA/600/R-99/029. National Risk Management Research Laboratory, U.S. Environmental Protection Agency. Cincinnati, Ohio. 443 pp. *(available at http://www.epa.gov/ednnrmrl/publish/book/epa-600-r-99-029).*

Pommepuy, M., J.F. Guillaud, E. Dupray, A. Derrien, F. Le Guyader and M. Cormier, 1992. Enteric bacteria survival factors. *Water Sci. Technol.*, 12: 93-103.

Powell, E.N., E.E. Hofmann, J.M. Klinck and S.M. Ray, 1992. Modeling oyster populations I. A commentary on filtration rate. Is faster always better?. *J. Shellfish Res.,* 11: 387-398.

Powell, E.N., J.M. Klinck, E.E. Hofmann and S.M. Ray, 1994. Modeling oyster populations. IV: Rates of mortality, population crashes and management. *Fish. Bull.*, 92: 347-373.

Prins, T.C., A.C. Smaal and R.F. Dame, 1998. A review of the feedbacks between bivalve grazing and ecosystem processes. *Aquat. Ecol.*, 31: 349-359.

Prins, T.C., A.C. Smaal, A.J. Pouwer and N. Dankers, 1996. Filtration and resuspension of particulate matter and phytoplankton on an intertidal mussel bed in the Oosterschelde estuary (SW Netherlands). *Mar. Ecol. Prog. Ser.,* 142: 121-134.

Prudente, M., H. Ichihashi, S. Kan-atireklap, I. Watanbe and S. Tanabe, 1999. Butyltins, organochlorines and metal levels in Green Mussel *Perna viridis* L. from the coastal waters of the Philippines. *Fish. Sci.,* 65 (3): 441-447.

Qasim, S.Z., 1978. Distribution of chlorophyll *a* in the Indian Ocean. *Indian J. Mar. Sci.,* 7: 258-262.

Qasim, S.Z. and R. Sengupta, 1981. Marine pollution studies in India. In: Varshney, C.K. (Ed.) Water Pollution and Management Reviews. South Asian Publishers Pvt. Ltd., Madras, 139-159.

Qasim, S.Z., S. Wellershaus, P.M.A. Bhattathiri and S.A.H. Abidi, 1969. Organic production in tropical estuary. *Proceedings of the Indian Academy of Science,* 69: 51-94.

Qasim, S.Z., A.H. Parulekar, S.N. Harkantra, Z.A. Ansari and A. Nair, 1977. Aquaculture of green mussel *Mytilus viridis* L. cultivation on ropes from floating rafts. *Indian J. Mar. Sci.,* 4: 189-197.

Quayle, D.B. and G.F. Newkirk, 1989. Farming bivalve mollusc: Methods for study and development. The World Aquaculture Society and International Development Research. Louisiana State University, Baton Rouge, LA., 294 pp.

Radhakrishnan, A.G., 1994. Organochlorine pesticide residues in farmed prawn (*Penaeus monodon*), oyster (*Crassostrea madrasensis*) and mussel (*Perna viridis*). In: Proceedings of the Symposium Nutrients and Bioactive Substances in Aquatic Organism. Society of Fisheries Technologists (India), Cochin, 266-270.

Radhakrishnan, A.G., 1994. Studies on the trace metal content of fish and shellfish including bivalves. In: Proceedings of the Symposium Nutrients and Bioactive Substances in Aquatic Organism. Society of Fisheries Technologists (India), Cochin, 271-275.

Radhakrishnan, A.G., P.D. Antony, M.K. Mukundan and J. Stephan, 1986. On the use of mussels as a pollution indicator. In: Proceedings of National seminar on mussel watch, Cochin, 23-32.

Rainbow, P.S., 1995. Biomonitoring of heavy metal availability in the marine environment. *Mar. Pollut. Bull.*, 31(4-12): 183-192.

Rajagopal, S., V.P. Venugopalan, G. Van der Velde and H.A. Jenner, 2006. Greening of the coasts: a review of the *Perna viridis* success story. *Aquat. Ecol.*, 40: 273-297.

Rajagopal, S., V.P. Venugopalan, K.V.K. Nair, G. Van der Velde and H.A. Jenner, 1998a. Settlement and growth of the green mussel *Perna viridis* (L.) in coastal waters: influence of water velocity. *Aquat. Ecol.*, 32: 313-322.

Rajagopal, S., V.P. Venugopalan, K.V.K. Nair, G. Van der Velde, H.A. Jenner and C. Den Hartog, 1998b. Reproduction, growth rate and culture potential of the green mussel, *Perna viridis* (L.) in Edaiyur backwaters, east coast of India. *Aquaculture,* 162: 187-202.

Rajagopalan, M.S., P.A. Thomas, K.J. Mathew, G.S.D. Selvaraj, R.M. George, C.V. Mathew, T.S. Naomi, P.Kaladharan, V.K. Balachandran and G. Antony, 1992. Productivity of the Arabian Sea along the Southwest coast of India. *Bull. Cent. Mar. Fish. Res. Inst.*, 45: 9-37.

Rajan, A., B. Shanthi and M. Kalyani, 1991. Effect of season, sex and reproduction on zinc concentration in the soft tissues of *Meretrix casta* (Chemnitz) (Molluscs: Bivalvia) collected from Vellar Estuary, Porto Nova, India. *Cienc. Mar.*, 17(2): 37-46.

Rajendran, N., J. Tagore and R. Kasinathan, 1988. Heavy metal concentrations in oyster, *Crassostrea madrasensis* (Preston) of Cuddalore backwaters, southeast coast of India. *Indian J. Mar. Sci.*, 17: 174-175.

Ramaiah, N., V. Kolhe and A. Sadhasivan, 2004. Abundance of pollution indicator and pathogenic bacteria in Mumbai waters. *Curr. Sci.*, 87(4) 435-439.

Ramamirtham, C.P. and D.S. Rao, 1974. On upwelling along the west coast of India. *J. Mar. Biol. Ass. India,* 15(1): 306-317.

Ramamirtham, C.P. and M.R. Patil, 1964. Hydrography of the west coast of India during the pre-monsoon period of the year 1962-Part 2: in and offshore waters of the Konkan and Malabar coasts. *J. Mar. Biol. Ass. India,* 7(1): 150-168.

Ramesh, A., S. Tanabe, A.N. Subramanian, D. Mohan, V.K. Venugopalan and R. Tatsukawa, 1990. Persistent organochlorine residues in green mussels from coastal waters of south India. *Mar. Pollut. Bull.*, 21 (12): 587-590.

Ramesha, A.M., 1989. Distribution of copepods and copepodites in relation to phytoplankton and hydrography of the coastal waters of Mangalore. M.F.Sc. Thesis Univ. Agril. Sci., Bangalore, 193 pp.

Ramesha, A.M., R.J. Katti, V.Hariharan, C. Bhat and T.R.C. Gupta, 1992. Phytoplankton of the coastal waters of Mangalore. *Environ. Ecol.,* 10(2): 310-316.

Rao, D.S., C.P. Ramamirtham, A.V.S. Murty, S. Muthusamy, N.P. Kunhikrishnan and L.R. Khambadkar, 1992. Oceanography of the Arabian Sea with particular reference to the southwest monsoon. *Bull. Cent. Mar. Fish. Res. Inst.*, 45: 4-8.

Rao, K.V., L.K. Kumari and S.N. Dwevedi, 1975. Biology of the green mussel, *Mytilus viridis*. *Indian J. Mar. Sci.,* 4: 189-197.

Rao, M., 1985. Distribution of suspended particulate matter in the waters of eastern continental margin of India. *Indian J. Mar. Sci.,* 14: 15-19.

Raveendran, O., P.S. Gore, T.S.G. Iyer, P.R.G. Varma and V.N. Sankaranarayanan, 1990. Occurrence of enteric bacteria in seawater and mussels along the southwest coast of India. *Indian J. Mar. Sci.*, 19: 282-284.

Ray, S.M and K.S. Rao, 1984. Manual on marine toxins in bivalve molluscs and general consideration of shellfish sanitation. *Cent. Mar. Fish. Res. Inst.*, Spl. Publn. No:16, 100 pp.

Reddy, H.R.V. and V. Hariharan, 1986. Distribution of total nitrogen, total phosphorus and organic carbon in the sediments off Mangalore. *Mahasagar: Bull. Natl. Inst. Oceanogr.*, 19(2): 119-122.

Rees, J.G., D. Setiapermana, V.A. Sharp, J. M. Weeks and T.M. Williams, 1999. Evaluation of the impacts of land-based contaminants on the benthic faunas of Jakarta Bay, Indonesia *Oceanol. Acta*, 22(6): 627-640.

Regoli, F., 1998. Trace metals and antioxidant enzymes in gills and digestive gland of the Mediterranean mussel *Mytilus galloprovincialis. Arch. Environ. Contam. Toxicol.*, 34: 48-63.

Regoli, F. and E. Orlando, 1994a. Seasonal variation of trace metal concentrations in the digestive gland of the mediterranean mussel *Mytilus galloprovincialis*: comparison between a polluted and a non-polluted site. *Arch. Environ. Contam. Toxicol.*, 27: 36-43.

Regoli, F. and E. Orlando, 1994b. Accumulation and subcellular distribution of metals (Cu, Fe, Mn, Pb and Zn) in the mediterranean mussel *Mytilus galloprovincialis* during a field transplant experiment. *Mar. Pollut. Bull.*, 28 (10): 592-600.

Reiriz, F.M.J., U. Labarta and J.M.F. Babarro, 1996. Comparative allometries in growth and chemical composition of mussel (*Mytilus galloprovincialis* Lmk) cultured in two zones in the Ria Sada (Galacia, NW Spain). *J. Shellfish Res.*, 15: 349-353.

Richards, G.P., 1978. Comparative study of methods for the enumeration of total and faecal coliforms in the eastern oyster, *Crassostrea virginica. Appl. Environ. Microbiol.*, 36(6): 975-978.

Ringwood, A.H., D.E. Conners and J. Hoguet, 1998. Effects of natural and anthropogenic stressors on lysosomal destabilization in oysters *Crassostrea virginica. Mar. Ecol. Prog. Ser.*, 166: 163-171.

Rivonker, C.U. and N. Verlecar, 1990. Physico-chemical characteristics of fishing grounds off Mangalore, west coast of India. *Indian J. Mar. Sci.*, 19: 201-205.

Rivonker, C.U. and A.H. Parulekar, 1998. Seasonal variation of major elements (Ca, Mg) and trace metals (Fe, Cu, Zn, Mn) in cultured mussel *Perna viridis* L. and seawater in the Dona Paula Bay, Goa. *Indian J. Mar. Sci.*, 27: 411-415.

Rivonker, C.U., Z.A. Ansari and A.H. Parulekar, 1993. Cultivation of green mussel, *Perna viridis* L., on a floating raft in an estuary along the west coast of India. *Aquaculture*, 112, 47-56.

Robisch, P.A. and R.C. Clark, 1993. Sample preparation and analyses of trace metals by atomic absorption spectroscopy. In Lauenstein, G.G. and A.Y. Cantillo (Eds.) Sampling and analytical methods of the National Status and Trends Program National Benthic Surveillance Program and Mussel Watch Project 1984-1992. Vol. III. Comprehensive description of elemental analytical methods. *NOAA Tech. Memo., NOS ORCA* 71, 111-150.

Rodhouse, P.G., C.M. Roden, G.M. Burnell, M.P. Hensey, T. Mc Mahon, B. Ottway and T.H. Ryan, 1984. Food source, gametogenesis and growth of *Mytilus edulis* on the shore and in suspended culture: Killary Harbour, Ireland. *J. Mar. Biol. Assoc. UK.*, 64: 513-529.

Ros, D.L., F. Meneghetti and C. Nasci, 2002. Field application of lysosomal destabilisation indices in the mussel *Mytilus galloprovincialis*: biomonitoring and transplantation in the Lagoon of Venice (north-east Italy). *Mar. Environ. Res.*, 54(3-5): 817-822.

Rosenberg, R. and L.O. Loo, 1983. Energy flow in a *Mytilus edulis* culture in western Sweden. *Aquaculture,* 35: 151-161.

Row, A., 1981. Microbiological studies in the Mandovi-Zuari River system. *Indian J. Mar. Sci.,* 10: 189-191.

Ruckelshaus, M.H., R.C. Wissmar and C.A. Simenstad, 1993. The importance of autotroph distribution to mussel growth in a well mixed temperate estuary. *Estuaries,* 16: 898-912.

Rylander, K., J. Pérez and J. Gómez, 1996. The distribution of the brown mussel, *Perna perna* and the green mussel, *P. viridis* (Mollusca: Bivalvia: Mytilidae) in North eastern Venezuela. *Caribb. Mar. Stud.*, 5: 86-87.

Sankar, T.V., A.A. Zynudheen, R. Anandan and P.G.V. Nair, 2006. Distribution of organochlorine pesticides and heavy metal residues in fish and shellfish from Calicut region, Kerala, India. *Chemosphere*, 65(4): 583-590.

Sankaranarayanan, V.N., K.S. Purushan and T.S.S. Rao, 1978. Concentration of some of the heavy metals in the oyster *Crassostrea madrasensis* from the Cochin region. *Indian J. Mar. Sci.,* 7: 130-131.

Sara, G. and A. Mazzola, 1997. Effects of trophic and environmental conditions on the growth of *Crassostrea gigas* in culture. *Aquaculture*, 153: 81-91.

Sara, G., A. Manganaro, G. Cortese, A. Pusceddu and A. Mazzola, 1998. The relationship between food availability and growth in *Mytilus galloprovincialis* in the open sea (southern Mediterranean). *Aquaculture*, 167: 1-15.

Sarkar, A. and R. Sengupta, 1989. Determination of organochlorine pesticides in Indian coastal water using a moored in-situ sampler. *Water Res.*, 23: 975-978.

Sarkar, A., R. Nagarajan, S. Chaphadkar, S. Pal and S.Y.S. Singbal, 1997. Contamination of organochlorine pesticides in sediments from the Arabian Sea along the west coast of India. *Water Res.*, 31(2): 195-200.

Sasikumar, G., P.K. Krishnakumar and G.S. Bhat, 2006. Monitoring trace metal contaminants in green mussel, *Perna viridis* from the coastal waters of Karnataka, Southwest coast of India. *Arch. Environ. Contam and Toxicol.,* 51(2): 206-214.

Sastry*, A.N., 1975. Physiology and ecology of reproduction in marine invertebrates. In: F.J. Vernberg (Ed.) Physiological Ecology of Estuarine Organisms, Univ. S. Carolina Press. Columbia, 279-299.

Sathyendranath, S., V. Stuart, B.D. Irwin, H. Maass, G. Savidge, L. Gilpin and T. Platt, 1999. Seasonal variations in bio-optical properties of phytoplankton in the Arabian Sea. *Deep-Sea Res. Part 2, Top. Stud. Oceanogr.*, 46(3-4): 633-653.

Saxby, S.A. 2002. A review of food availability, sea water characteristics and bivalve growth performance at coastal culture sites in temperate and warm temperate regions of the world. Fisheries Research Report No. 132, 42 pp.

Schiedek, D., K. Broeg, J. Barsien, K.K. Lehtonen, J. Gercken, S. Pfeifer, H. Vuontisjarvi, P.J. Vuorinen, V. Dedonyte, A. Koehler, L. Balk and R. Schneider, 2006. Biomarker responses as indication of contaminant effects in blue mussel (*Mytilus edulis*) and female eelpout (*Zoarces viviparus*) from the southwestern Baltic Sea. *Mar. Pollut. Bull.,* 53: 387-405.

Schueler, T.R., 2000. Microbes and Urban watersheds: Concentrations, Sources and Pathways. In: T.R. Schueler and H.K. Holland, (Eds.) *The Practice of Watershed Protection*, Center for Watershed Protection. Ellicott City, Maryland, 74-84. *(http://www.polytechnic.edu.na/Schools/civil/Libraries/Hydrology/17-microbes%20in%20Urban%20Watersheds.pdf)*

Seed, R., 1976. Ecology. In: Bayne B.L. (Ed.) Marine Mussels: Their Ecology and Physiology, Cambridge University Press, Cambridge, 13-65.

Seed, R. and T.H. Suchanek, 1992. Population and community ecology of *Mytilus*. In: Gosling E. (Ed.) The mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier Science, Amsterdam, 87-169.

Segar, K., 1982. Nitrogen cycle and its influence on the standing crop of plankton in a fertilizer effluent discharge area off Mangalore. M.F.Sc. Thesis Univ. Agril. Sci., Bangalore, 203 pp.

Segnini, de Bravo, M.I., Chung, K.S. and Perez, J.E. 1998. Salinity and temperature tolerances of the green and brown mussels, *Perna viridis* and *Perna perna* (Bivalvia, Mytilidae). *Revista de Biologia Tropical*, Supplement, 46(5): 121-126.

Selvan, K. and V.K. Pillai, 1988. A study on the bacterial quality of brown mussel *Perna indica* and its purification. In National Seminar on shellfish resources and farming, Part-II, *Bull. Cent. Mar. Fish. Res. Inst.*, 42: 431-435.

Selvaraj, G.S.D., V.J. Thomas and L.R. Khambadkar, 2003. Seasonal variation of phytoplankton and productivity in the surf zone and backwater at Cochin. *J. Mar. Biol. Ass. India,*, India, 45(1): 9-19.

Sengupta, R., S.Y.S. Singbal and S. Sanzgiri, 1978. Atomic absorption analysis of a few trace metals in Arabian Sea waters. *Indian J. Mar. Sci.*, 7: 295-299.

Senthilnathan, S. and T. Balasubramanian, 1998. Heavy metal concentration in oysters, *Crassostrea madrasensis* (Bivalvia/Anisomyaria) from the Uppanar, Vellar and Kaduviar Estuaries of southeast coast of India. *Indian J. Mar. Sci.*, 27(2): 211-216.

Senthilnathan, S., T. Balasubramanian and V.K. Venugopalan, 1998. Metal concentration in mussel, *Perna viridis* (Bivalvia/ Anisomyaria) and Oysters, *Crassostrea madrasensis* (Bivalvia/ Anisomyaria) from some parts in southeast coast of India. *Indian J. Mar. Sci.*, 27(2): 206-210.

Shafee, M.S., 1979. Ecological energy requirements of the green mussel, *Perna viridis* Linnaeus from Ennore estuary, Madras. *Oceanol. Acta* 2(1): 69-74.

Shankar, D., S.S.C. Shenoi, R.K. Nayak, P.N. Vinayachandran, G. Nampoothiri A.M. Almeida, G.S. Michael, M.R. Rameshkumar, D. Sundar and O.P. Sreejith, 2005. Hydrography of the eastern Arabian Sea during summer monsoon 2002. *J. Earth Syst. Sci.*, 114(5): 459-474.

Shepard, J.L. and B.P. Bradley, 2000. Protein expression signatures and lysosomal stability in *Mytilus edulis* exposed to graded copper concentrations. *Mar. Environ. Res.*, 50(1-5): 457-463.

Shi, D. and W.X. Wang, 2004. Modification of trace metal accumulation in the green mussel *Perna viridis* by exposure to Ag, Cu and Zn. *Environ. Pollut.*, 132(2): 265-277.

Shieh, Y.C., R.S. Baric, J.W. Woods and K.R. Calci, 2003. Molecular surveillance of enterovirus and norwalk-like virus in oysters relocated to a municipal-sewage-impacted Gulf Estuary. *Appl. Environ. Microbiol.*, 69(12): 7130-7136.

Shin, P.K.S., F.N. Yau, S.H. Chow, K.K. Tai and S.G. Cheung, 2002. Responses of the green-lipped mussel *Perna viridis* (L.) to suspended solids. *Mar. Pollut. Bull.*, 45: 157-162.

Shuhong, W., H. Huasheng and W. Xinhong, 2005. Bioenergetic responses in green lipped mussels (*Perna viridis*) as indicators of pollution stress in Xiamen coastal waters, China. *Mar. Pollut. Bull.*, 51: 738-743.

Shumway, S.E., 1992. Mussels and public health. In: Gosling E. (Ed.) The mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier Science, Amsterdam, 511-540.

Siddall, S.E., 1980. A clarification of the genus *Perna* (Mytilidae). *Bull. Mar. Sci.*, 30(4): 858-870.

Sinton, L.W., R.J.D. Colley and R.G. Bell, 1994. Inactivation of enterococci and faecal coliforms from sewage and meatworks effluents in seawater chambers. *Appl. Environ. Microbiol.*, 60(6): 2040-2048.

Sinton, L.W., R.K. Finlay and P.A. Lynch, 1999. Sunlight inactivation of faecal bacteriophages and bacteria in sewage-Polluted seawater *Appl. Environ. Microbiol.*, 65(8): 3605-3613.

Sivalingam, P.M. 1977. Aquaculture of the green mussel, *Mytilus viridis* Linnaeus, in Malaysia, *Aquaculture*, 11: 297-312.

Sivalingam, P.M., 1984. Ocean disposal and land reclamation problems of Penang, Malaysia. *Conserv. Recy.*, 7(2-4): 85-98.

Smaal, A.C. and M.R. van Stralen, 1990. Average annual growth and condition of mussels as a function of food source. *Hydrobiologia*, 195: 179-188.

Smaal, A.C. and H.A. Haas, 1997. Seston dynamics and food availability on mussel and cockle beds. *Est. Coast. Shelf Sci.*, 45: 247-259.

Smaal, A.C., J.H.G. Verhagen, J. Coosen and H.A. Haas, 1986. Interaction between seston quantity and quality and benthic suspension feeders in the Oosterschelde, The Netherlands. *Ophelia*, 26: 385-399.

Son, N.T. and G.H. Fleet, 1980. Behavior of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, re-laying and storage. *Appl. Environ. Microbiol.* 40(6): 994-1002.

Soto, M., M. Kortabitarte and I. Marigomez, 1995. Bioavailable heavy metals in estuarine waters as assessed by metal/shell-weight indices in sentinel mussels *Mytilus galloprovincialis. Mar. Ecol. Prog. Ser.*, 125: 127-136.

Sphigel, M., B.J. Barber and R. Mann, 1992. Effects of elevated temperature on growth, gametogenesis, physiology and biochemical composition in diploid and triploid Pacific oysters, *Crassostrea gigas* Thunberg. *J. Exp. Mar. Biol. Ecol.*, 161: 15-25.

Stirling, H.P. and I. Okumus, 1995. Growth and production of mussels (*Mytilus edulis* L.) suspended at salmon cages and shellfish farms in two Scottish sea lochs. *Aquaculture*, 134: 193-210.

Strickland, J.D.H. and T.R. Parsons, 1968. A practical handbook of seawater analysis. *Bull. Fish. Res. Board Can.*, 167, 311 pp.

Strickland, J.D.H. and T.R. Parsons, 1972. A practical handbook of seawater analysis 2. ed. *Fish. Res. Bd. Can. Bull.*, Ottawa, Canada, 311 p.

Subrahmanyan, R., 1959. Studies on the phytoplankton of the west coast of India. *Proc. Indian Acad. Sci.*, 50 B: 113-252.

Subrahmanyan, R., 1969. Fisheries Oceanography in India. *Indian Farming*, 19(9): 47-52.

Subramanian, B. and A. Mahadevan, 1999. Seasonal and diurnal variation of hydrobiological characters of coastal water of Chennai (Madras), Bay of Bengal. *Indian J. Mar. Sci.*, 28: 429-433.

Suchanek, T.H., 1978. The ecology of *M. edulis* L. in exposed rocky intertidal communities. *J. Exp. Mar. Biol. Ecol.*, 31: 105-120.

Suchanek, T.H., 1985. Mussels and their role in structuring rocky shore communities. In: P.G. Moore and R. Seed (Ed.) The Ecology of Rocky coasts. Hodder and Stoughton, Sevenoaks, U.K., 70-76.

Sukasem, P. and M.S. Tabucanon, 1993. Monitoring heavy metals in the Gulf of Thailand using mussel watch approach. *Sci. Total Environ.*, 139-140: 297-305.

Summer, A.T. 1969. The distribution of some hydrolytic enzymes in the cells of the digestive gland of certain lamellibranches and gastropods. *J. Zool. London*. 158: 277-291.

Sundaram, K.S. and M.S. Shafee, 1989. Salinity tolerance of some bivalves of Ennore estuary. *J. Mar. Biol. Ass. India*, 31: 299-302.

Sunil, R., I. Karunasagar and I. Karunasagar, 2004. Study of bacteriological quality of water in aquatic habitats of Karnataka Coast. Conference on Microbiology of the Tropical Seas. National Institute of Oceanography, Dona Paula, Goa 403 004. India, 13-15 December 2004. Abstract HPM (P)-01.

Surendran, P.K. and K.K. Balachandran, 1988. Removal of pathogenic bacteria and grittiness from clam (*Villorita cyprinoides*) and mussel (*Perna viridis*) meant for processing by a biological method. In National Seminar on shellfish resources and farming, Part-II, *Bull. Cent. Mar. Fish. Res. Inst.*, 42: 404-409.

Surendran, P.K., N. Thampuran, V.N. Nambiar and K.V. Lalitha, 2002. Microbial ecology of inland water bodies with special reference to fisheries. In: Boopendranath, M.R., Meenakumari, B., Joseph, J., Sankar, T.V., Pravin P. and Edwin L., (Eds.) Riverine and Reservoir Fisheries of India. Society of Fisheries Technologist (India), Cochin, 6-14.

Suresh, K. and A. Mohandas, 1990. Hemolymph acid phosphatase activity pattern in copper-stressed bivalves. *J. Invertebr. Pathol.* 55: 118-125.

Suresh, K., M.P.M. Reddy and N.P. Kurian, 1978. Hydrographic features of near-shore waters along Mangalore coast. *Indian J. Mar. Sci.*, 7:141-145.

Sze, P.W.C. and S.Y. Lee, 1995. The potential role of mucus in the depuration of copper from the mussels *Perna viridis* (L.) and *Septifer virgatus* (Wiegmann) *Mar. Pollut. Bull.*, 31(4-12): 390-393.

Szefer, P., B.S. Kim, C.K. Kim, E.H. Kim and C.B. Lee, 2004. Distribution and coassociations of trace elements in soft tissue and byssus of *Mytilus galloprovincialis* relative to the surrounding seawater and suspended matter of the southern part of Korean Peninsula. *Environ. Pollut.*, 129: 209-228.

Tan, S.H., 1997. Effect of salinity on hatching, larval growth and survival in the green mussel *Perna viridis* (Linnaeus). *Special Publication of the Phuket Mar. Biol. Center*, 1: 279-284.

Tan, W.H. and L.H. Lim, 1984. The tolerance to and uptake of lead in the green mussel, *Perna viridis* (L.) *Aquaculture*, 42(3-4): 317-332.

Tanabe, S., 1994. International Mussel Watch in Asia-Pacific Phase (Editorial). *Mar. Pollut. Bull.*, 28(9): 518.

Tanabe, S. M.S. Prudente, S. Kan-atireklap and A. Subramanian, 2000. Mussel watch: marine pollution monitoring of butylins and organochlorines in coastal waters of Thailand, Philippines and India *Ocean Coast. Manage.*, 43(8-9): 819-839.

Tedengren, M. and N. Kautsky, 1986. Comparative studies on the physiology and its probable effect on size in blue mussels (*Mytilis edulis*) from the North Sea and Northern Baltic proper. *Ophelia*, 25: 147-155.

Tewari, A., H.V. Joshi, C. Raghunathan, V.G. Sravankumar and O.S. Kotiwar, 2000. New record of *Mytilus viridis* Linn., its density, growth and accumulation of heavy metals on Saurashtra coast, Arabian sea. *Cur. Sci.*, 78: 97-102.

Tewari, A., H.V. Joshi, C. Raghunathan, V.G. Sravankumar and Y. Khambhaty, 2001. Effect of heavy metal pollution on growth, carotenoid content and bacterial flora in the gut of *Perna viridis* (L.) in *in situ* condition. *Curr. Sci.*, 81: 819-828.

Thippeswamy, S. 1990. Community ecology of the green mussel *Perna viridis* (L) island microhabitat. Ph.D Thesis, Univ. Agril. Sci., Bangalore, 235pp.

Thompson, J.F., S.J. Reid and E.J. Kantor, 1977. Multiclass, multiresidue analytical method for pesticide in water. *Arch. Environ. Contam. Toxicol.*, 6(2-3): 143-157.

Thorarinsdottir, G.G., 1994. The Iceland scallop, *Chlamys islandica* (O.F. Mueller), in Breidafjoerdur, west Iceland. 3. Growth in suspended culture. *Aquaculture*, 120: 295-303.

Toro, J.E. 1985. Annual cycle and composition of the phytoplankton in the Quempillen river estuary, southern Chile. *Est. Coast. Shelf Sci.*, 21: 25-34.

Toro, J.E., P.I. Paredes and D.J. Villagra, 1999. Phytoplankton distribution and oyster, *Ostrea chilensis* (Philippi 1845) growth at Putemŭn Channel, southern Chile. *N. Z. J. Mar. Freshw. Res.*, 33: 499-513.

Tortorello, M.L., 2003. Indicator organisms for safety and quality-uses and methods for detection: minireview. *J. AOAC Int.*, 86(6): 1208-1217.

Tree, J.A., M.R. Adams and D.N. Lees, 2003. Chlorination of indicator bacteria and viruses in primary sewage effluent. *Appl. Environ. Microbiol.*, 69(4): 2038-2043.

Tsuchiya, M., 1983. Mass mortality in a population of the mussel *Mytilus edulis* L. caused by high temperature on rocky shores. *J. Exp. Mar. Biol. Ecol.*, 66: 101-111.

Urban, E.R., D.L. Kirchman, 1992. Effect of kaolinite clay on the feeding activity of the eastern oyster *Crassostrea virginica* (Gmelin). *J. Exp. Mar. Biol. Ecol.*, 160: 47-60.

Urrutia, M.B., I. Ibarrola, J.I.P. Iglesias and E. Navarro, 1999. Energetics of growth and reproduction in a high-tidal population of the clam *Ruditapes decussatus* from Urdabai Estuary (Basque Country, N. Spain). *J. Sea Res.*, 42: 35-48.

Vahl, O., 1980. Seasonal variations in seston and the growth rate of the Iceland scallop, *Chlamys islandica* (O.F. Muller) from Balsfjord, 70°N. *J. Exp. Mar. Biol. Ecol.*, 48: 195-204.

Vakily, J.M., 1992. Determination and comparison of bivalve growth, with emphasis on Thailand and other tropical areas. *ICLARM Tech. Rep.* 36, 125 pp.

van den Broek, M.J.M., D.A.A. Mossel and A.E. Eggenkamp, 1979. Occurrence of *Vibrio parahaemolyticus* in Dutch mussels. *Appl. Environ. Microbiol.*, 37(3): 438-442.

van Dolah, R.F, D.E. Chestnut and G.I. Scott, 2000. A Baseline Assessment of Environmental and Biological Conditions in Broad Creek and the Okatee River, Beaufort County, South Carolina. Prepared by South Carolina Department of Health and Environmental Control, South Carolina Department of Natural Resources and National Oceanic and Atmospheric Administration, National Ocean Service. Final Report to the Beaufort County Council. Beaufort County, South Carolina, 115 pp. (*available at http://www.scdhec.gov/eqc/water/html/broadokateerp/title.html*).

Varkey, M.J., 2005. Development of summer monsoon and onset of continuous rains over central west coast of India. Proceedings of National Symposium on Half A Century Progress in Oceanographic studies of North Indian Ocean since Prof. La Fond's Contributions, HACPO, 38-45.

Venkataraman, R. and A. Sreenivasan, 1955. Mussel pollution at Korapuzha Estuary (Malabar) with an account of certain coliform types. *J. Indian Fish.*, 2: 314-324.

Verlecar, X.N. and S.Z. Qasim, 1985. Particulate organic matter in the coastal and estuarine waters of Goa and its relationship with phytoplankton production. *Est. Coast. Shelf Sci.*, 21: 235-243.

Verlecar, X.N., S.R. Desai, A. Sarkar and S.G. Dalal, 2006. Biological indicators in relation to coastal pollution along Karnataka coast, India. *Water Res.*, 40: 3304-3312.

Villalba, A., 1995. Gametogenic cycle of cultured mussel, *Mytilus galloprovincialis*, in the bays of Galicia (N.W.Spain). *Aquaculture*, 30: 269-277.

Villela, P.C.A., 1984. Effect of reduced salinity on the shell growth of small *Mytilus edulis*. *J. Mar. Biol. Ass. U.K.*, 64: 171-182.

Walne*, P.R. 1970. The seasonal variation of meat and glycogen content of seven populations of oyster *Ostrea edulis* L. and a review of the literature. *Min. Agric. Fish. Food. Fish. Invest., Ser.*, 2: 26(3).

Walne*, P.R. and R. Mann, 1975. Growth and biochemical composition in *Ostrea edulis* and *Crassostrea gigas*. In: Barnes, H. (Ed.) *Proc. 9th Europ. Mar. Biol. Symp.* Aberdeen Univ. Press, 587-607.

Walter, C. 1982. Reproduction and growth in the tropical mussel, *Perna viridis* (Bivalvia: Mytilidae), Kalikasan. *Philipp. J. Biol.*, 11: 83-97.

Wang, W.X. and R.C.K. Wong, 2003. Combined effects of food quantity and quality on Cd, Cr and Zn assimilation to the green mussel, *Perna viridis*. *J. Exp. Mar. Biol. Ecol.*, 290(1): 49-69.

Ward, J.E. and B.A. MacDonald, 1996. Pre-ingestive feeding behaviours of two sub-tropical bivalves (*Pinctada imbricata* and *Arca zebra*): Responses to an acute increase in suspended sediment concentration. *Bull. Mar. Sci.*, 59: 417-432.

Weiskel, P., Howes, B. and G. Heufelder, 1996. Coliform contamination of a coastal embayment: sources and transport pathways. *Environ. Sci. Technol.*, 30(6): 1872-1881.

White, N.M., D.E. Line, J.D. Potts, W.K. Smith, B. Doll and W.F. Hunt, 2000. Jump Run Creek shellfish restoration Project. *J. Shellfish Res.*, 19(1): 473-476.

WHO (World Health Organisation), 1972. Evaluation of certain food additives and the contaminants Mercury, Lead, Cadmium. 16th Report of the Joint FAO/WHO Expert Committee on Food Additives. Technical Report Series 505, Geneva.

WHO (World Health Organisation), 1987. Evaluation of certain food additives and contaminants. Thirty-third report of the joint FAO/WHO expert committee on food additives. WHO Technical Report Series 776, Geneva.

Widdows, J., 1991. Physiological ecology of mussel larvae. *Aquaculture*, 94, 147-163.

Widdows, J. and Donkin, P., 1992. Mussels and environmental contaminants: bioaccumulation and physiological aspects. In: Gosling E. (Ed.) The mussel Mytilus: Ecology, Physiology, Genetics and Culture. Elsevier Science, Amsterdam, 383-424.

Widdows, J., P. Fieth and C.M. Worrall, 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Biol.*, 50: 195-207.

Williams, P., 1981. Detritus utilization by *Mytilus edulis*. *Estuar. Coast. Shelf Sci.*, 12: 739-746.

Wilson, J.H., 1987. Mussels: The problem of early spawning. *Aquacult. Ir.*, 30: 20-21.

Wong, C.K.C., R.Y.H. Cheung and M.H. Wong, 2000. Heavy metal concentrations in green-lipped mussels collected from Tolo Harbour and markets in Hong Kong and Shenzhen. *Environ. Pollut.*, 109(1): 165-171.

Wong, W.H. and S.G. Cheung, 2001. Feeding behaviour of the green mussel, *Perna viridis* (L.): Responses to variation in seston quantity and quality. *J. Exp. Mar. Biol. Ecol.*, 236: 191-207.

Wood, P.C., 1972. The principles and methods employed for the sanitary control of molluscan shellfish. In: M. Ruivo (Ed.) Marine Pollution and Sea Life. Fishing News (Books) Ltd., England, 560-565.

Wu, Y., J. Zhang and Q. Zhou, 1999. Persistent organochlorine residues in sediments from Chinese river/ estuary systems *Environ. Pollut.*, 105(1): 143-150.

Yap, C.K., A. Ismail, S.G. Tan and H. Omar, 2002. Correlations between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. *Environ. Int.*, 28(1-2): 117-126.

Yap, C. K., A. Ismail, S.G. Tan and I.A. Rahim, 2003a. Can the shell of the green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn?. *Est. Coast. Shelf Sci.*, 57(4): 23-630.

Yap, C.K., A. Ismail and S.G. Tan, 2003b. Can the byssus of green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a biomonitoring organ for Cd, Pb and Zn? Field and laboratory studies. *Environ. Int.*, 29(4): 521-528.

Yap, C.K., A. Ismail and S.G. Tan, 2004a. Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. *Food Chem.*, 84(4): 569-575.

Yap, C.K., A. Ismail, H. Omar and S.G. Tan, 2004b. Toxicities and tolerances of Cd, Cu, Pb and Zn in a primary producer (*Isochrysis galbana*) and in a primary consumer (*Perna viridis*). *Environ. Int.*, 29(8): 1097-1104.

Yap, C.K., A. Ismail, W.H. Cheng and S.G. Tan, 2006. Crystalline style and tissue redistribution in *Perna viridis* as indicators of Cu and Pb bioavailabilities and contamination in coastal waters. *Ecotoxicol. Environ. Saf.*, 63(3): 413-423.

Young, K.D. and E.L. Thackston, 1999. Housing density and bacterial loading in urban streams. *J. Environ. Eng.*, 125(12): 1177-1180.

Yung, Y.K., C.K. Wong, K. Yau and P.Y. Qian, 2001. Long-term changes in water quality and phytoplankton characteristics in Port Shelter, Hong Kong, from 1988-1998. *Mar. Pollut. Bull.*, 42(10): 981-992.

Zingde, M.D., S.Y.S. Singbal, C.F. Moraes and C.V.G. Reddy, 1976. Arsenic, copper, zinc and manganese in the marine flora and fauna of coastal and estuarine waters around Goa. *Indian J. Mar. Sci.*, 5: 212-217.

* not referred to in original.